PLANT PHYSIOLOGY

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Page 606, in formula for value of t, for "$x_1 - x_2$" read $\bar{x}_1 - \bar{x}_2$.

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Page 168, last line, for "Tierschen" read tierischen.
Page 189, fifth line from bottom, for "suelement" read seulement.
Page 199, citation 67, for "psychometric" read psychrometric.
Page 201, citation 105, for "gegenseitiegen" read gegenseitigen.
Page 336, table I, last line, for "90" read 133.

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PLANT PHYSIOLOGY

JANUARY, 1933

SEASONAL ABSORPTION OF NUTRIENT SALTS BY THE FRENCH PRUNE GROWN IN SOLUTION CULTURES

HAROLD L. COLBY

(WITH EIGHT FIGURES)

Introduction

Splendid theoretical as well as practical work has been done by German scientists on seasonal absorption of nutrient salts from the soil by forest trees, both coniferous and dicotyledonous. As a result, the German foresters have found it desirable to increase the efficiency of the utilization of soil nitrate over the entire season, by employing the mixed type of forest planting, instead of planting pure stands of a single species of timber tree. Different species of forest trees have very different periods of nitrate absorption, ranging from the "early season" nitrate absorbers (the pines, etc.) to the "late fall" absorbers (the horse-chestnut, and others) (**32**).

No similar seasonal absorption studies have been made for any of our deciduous fruit trees. The practical importance of such studies in relation to commercial fertilization of orchard trees is obvious. The theoretical importance to a final understanding of fruit-tree physiology is perhaps of still greater importance. There is reason to believe that fruit trees obtaining a moderate supply of a given element early in the growing season, but severely starved for that element later in the season, will behave very differently from trees continuously starved, or from trees "fed" only late in the growing season or during the entire season. Seasonal absorption is partly inherent; that is, it is a characteristic of the variety, and partly subject to alterations in the soil solution and to other environmental changes.

Historical resumé

The course of nutrient absorption throughout the growing season by most of our field-crop plants has been well known for some time. Thorough studies were made on absorption by forage crops, grain crops, and root crops by such German workers as WOLFF (**56**), KNOP (**17**), SACHS (**41**),

LIEBSCHER (**21**), and others. Yet at the present time, apparently no work has been published on the seasonal absorption in fruit trees. Certainly no such work with trees grown in water culture has previously appeared; yet chemical analyses of particular parts of orchard trees, taken at various seasons of the year, are plentiful enough. The seasonal nutrition of forest trees is comparatively well understood, thanks to the rather recent efforts of the German foresters, KÜBLER (**20**), BAUER (**32**), RAMANN (**32**), GOSSNER (**35**), SÜCHTING (**44**), JOHN, DEINES, WEIDELT, and MARSHARD; and of COMBES (**8**) in France. From RAMANN, who has done most of the chemical work on trees at Munich, comes the following interesting statement: "Die Erkenntnis dass die Nahrstoffaufnahme der Baumarten zeitlich verschieden ist, halte ich fur sichergestellt."

It may be well to state here, that while no seasonal absorption studies in fruit trees have been made previously, yet general nutrition studies in citrus have been made by REED and HAAS (**36**), and in apple, plum, and small fruits by WALLACE (**49**) and MANN (**23**).

Since the present experiment deals entirely with water-culture work, it will be profitable to take a brief backward glance over the history of water-culture work with trees, and with field-crop plants. Curiously enough, although so little work has been done with trees growing in solutions, yet water-culture work with plants really began with the growing of *trees* in water! DUHAMEL DU MONCEAU (**10**) in his early work "Physique des Arbres" (part 2), describes the growth of almond, oak, and chestnut trees in the water of an open fountain in a garden in France, in 1758. The fountain water was from a filtered supply from the River Seine. His oak tree, growing from an acorn accidentally dropped into the fountain, grew for some eight years, with no other mineral nourishment than that supplied by the fountain. At the end of eight years the "tree" was 18 inches high. His almond tree grew well for four years in the same fountain. The chestnut tree apparently grew as well in water as in soil, and was planted in garden soil after two years in the fountain. DUHAMEL was convinced that only water was needed for plant growth; he did not know that his fountain of river-water carried dissolved salts. It is well to note here, that ARISTOTLE also held the idea (perhaps gathered from similar, but unrecorded observations) that water was transformed by living organisms into body tissues. Long afterward, VAN HELMONT thought that his famous willow tree took only water from the soil. DUHAMEL'S experiments and his conclusions widely influenced the thought on plant nutrition at the time, and up until 1804, when DE SAUSSURE finally corrected the errors of DUHAMEL'S work by growing plants in distilled water with and without added salts. DE SAUSSURE also used single-salt solutions, and demonstrated the differential absorption of salts by plants.

One hundred years later WOLFF and KNOP (**57**) grew oak trees in water culture (pure salt solutions) to an age of 15 years, probably a long-time record for such work. They placed their trees in river water during each winter, so that exact control of salts supplied to the trees was not attained. At the end of 15 years the oak tree was 1.64 meters high, and the stem circumference was about 5 cm. Fifteen-year-old forest oak trees were 8–9 meters high, with stem circumference of 30.4 cm.

NOBBE (**28**) had fair success with two-year-old (1 meter high) elm trees in water-culture solutions. He measured the transpiration of these trees and found that it was one-tenth as great during the night as during the day. He also found that "yellow" light apparently checked transpiration more than did "blue" light. NOBBE also found that the ash content of his water-culture trees was usually greater than the ash content of trees grown in soil.

BAIN (**1**) grew a number of peach and apple seedlings and grape vines in water solutions, studying the sensitivity of the roots to copper in the solution. Apple roots were found most sensitive, peach less so, and grape least sensitive to copper salts. Later REED (**36**) made use of water cultures in studying the nutrition of young citrus seedlings, etc. Recently COMBES (**8**) compared the chemical composition of beech trees grown in water cultures with the composition of similar trees grown in forest soil, and obtained some rather interesting results. To date, it must be admitted that in no case have trees grown in water culture made equal growth with trees grown in rich soil. Nor have trees ever been grown to full maturity in water-culture work, something that must be done before tree nutrition studies are placed on a plane with field-crop nutrition studies.

Apparently, then, what knowledge we have of the nutrition of deciduous fruit trees did not come from water-culture work. Instead, small tub sand cultures have been largely used, and orchard fertilizer plot experiments have been carried on in various parts of the world, for many years. Results from the latter type of experimentation may have local importance, but the findings are often misleading, and, because of complicated soil conditions, usually tell us little or nothing of the quantitative needs of the orchard tree. More light has been thrown on tree physiology through sand-culture methods, and through chemical analyses of tree tissue produced in cultures under varying conditions of nutrition, water supply, shading, length of day, etc.

In the field of sand cultures, with deciduous fruit trees, the work of MANN (**23**) and WALLACE (**49, 50**) is outstanding. WALLACE interested himself chiefly in the elemental starvation of young trees of apple and plum, and of strawberry and gooseberry plants, etc., among small fruits. He starved particular trees for single elements over periods of three consecutive years, noted differences in growth of roots, stems, and leaves, and

recorded differences in the ash content of various organs after such starvation treatments. He was able to produce characteristic symptoms of phosphorus, magnesium, or potassium starvation, etc., during the first season in which the particular element was withheld from the solution.

WALLACE also studied the combined effects of summer pruning in young trees with starvation for the various elements. However, he made no studies of seasonal absorption by any of his plants or trees.

ROBERTS (**39**) grew dwarf apple trees in sand cultures with high and low nitrogen, short and long-day conditions, etc., and concluded that the type of growth of a tree is a response to the balance of carbohydrate-nitrogen content, rather than to absolute amounts of these materials present in the tree. Also, trees growing in a high nitrogen culture were capable of storing enough nitrogen in one year to make a normal growth the following year when placed in a nitrogen-free culture.

As to the speed with which deciduous fruit trees can absorb particular ions, transport them to the leaves, and utilize them in synthesis in the leaf or elsewhere, KNOWLTON'S (**18**) work is interesting. He used the "half-tree" nitrogen fertilization method, with bearing apple trees, applying nitrate of soda to the ground under only one side of the tree as the fruit buds were just swelling in spring. Twelve days elapsed before an increase in total nitrogen was noticeable in the fruit buds on the nitrated portion of the tree. Later in the season, quicker response to nitrogen is expected. THOMAS (**46**) found that a nitrate application to soil on June 6 raised total nitrogen in one-year twigs within one week from the date of fertilization. Foliage color, of course, deepens at about the same rate in response to added nitrate nitrogen.

Seasonal absorption studies with forest trees

It will be interesting to review briefly the knowledge of seasonal absorption in forest trees. First of all, it may be noted that the ash content of both conifers and dicotyledonous trees varies with the elevation at which the trees are grown, and with the composition of the soil in which they grow. The ash content (in percentage of dry weight) and size of leaves (or needles) decrease very markedly with increased elevation (GRANDEAU **12**). However, the course of seasonal absorption may not be particularly different, except at very high altitudes, where a short growing season checks full plant development.

BAUER (**5**) found that apparently every species of forest tree has a different course of mineral-salt absorption throughout the year, as well as a different curve for dry-weight increase throughout the season. Frequently absorption and dry-weight increase follow closely along the same path, but this is not always the case, and probably never is there perfect correlation

between these two activities. The same tree may have very different seasonal absorption for various ions. Although nitrogen and potassium absorption curves usually run parallel, nitrogen and magnesium, or nitrogen and phosphorus curves may be very different. The fir tree absorbed its nitrogen before June 1 each year, in most cases, with no nitrogen absorption from June 1 to September 15. The horse-chestnut took in its nitrogen from June 1 to November 1, continuing a rapid absorption very late in the fall. Larch trees took in almost no nitrogen in June, but were absorbing potassium, calcium, magnesium, and phosphorus at that time. The maximum absorption rate for nitrogen in the ash tree fell in June; for the beech tree, in August. COMBES (**8**) reported the maximum absorption of nitrogen in the beech tree in July and in October, although he made no observations in August. According to COMBES, at the time of leaf fall there was a tremendous increase (50 per cent.) in the total nitrogen of the beech tree, not all of the increase being accounted for by the backward movement of nitrogen from the leaves.

Taking the ash tree as somewhat typical of the behavior of dicotyledonous trees, we find that in two-year-old trees (BAUER **4**), at the start of the experiment on February 27, the dry weight of the entire root system was equal to 2.2 times the weight of the stems. But by November of the same year (after a season's growth in forest soil) the weight of the stem was almost equal to the weight of the root. As to the absorption of nutrients for the entire tree, for the first period (February 27–May 21) the *dry-weight increase was negligible,* and absorption of potassium, nitrogen, magnesium, etc., was slow; but *the absorption of calcium was rapid,* and actual loss of phosphorus appears to have occurred. Iron absorption was fairly rapid, but reached its maximum rate in the second period (May 21–July 21), then slowed down in the third period (July 21–September 17), and from September 17 to November 17, severe loss (60 per cent.) of iron occurred from the entire plant. The stem lost in total iron in the first period; also in the last period. It must be admitted that iron analyses may not have been entirely accurate, owing partly to the small amounts of the element present in tree tissues.

With reference to nitrogen, both roots and stems lost in absolute total nitrogen in the first period; and the root lost nitrogen again in the second period, in spite of the fact that it had already started gaining in total potassium content, of which it lost 60 per cent. of the total initially present, during the first period. In the first period, also, the root of the ash tree lost magnesium, nitrogen, and phosphorus, and 32 per cent. of its dry weight, but *gained 9 per cent. in total calcium.* During the first period the stem lost only 1 per cent. of its dry weight, far less than that lost by the root. During the second period phosphorus was absorbed rapidly; then the total

phosphorus fell off to the end of the season. In the last period (September 17–November 17), the period of leaf-fall, the tree lost in total calcium, nitrogen, magnesium, iron, phosphorus, and dry weight; but total potassium remained practically constant. BAUER concluded that potassium must be absorbed rather rapidly right to the end of the season.

SÜCHTING (**44**) ET AL., working with beech and other forest trees, showed that during the winter period (September to January) the tree gained in total phosphorus (very markedly), in total nitrogen, and in total calcium, but lost slightly in potassium. SÜCHTING also compared absorption curves of trees with those of the potato; in both cases the start was made with a rather large storage organ, and in such plants the early spring absorption was *not dominated by potassium absorption,* as is the case in rye, etc., where only small amounts of stored food are present at the beginning of the season, in the seed. The contrast is at least interesting, in view of the possible importance of potassium in carbohydrate synthesis in the plant.

KÜBLER (**20**) experimented with two plots of young beech trees, one plot being given complete fertilizer and the other plot left unfertilized. In following the seasonal absorption in these two sets of trees, he found that the absorption curves were similar in the early periods (up to July), but from July to September the fertilized trees had far more rapid absorption, and increased in dry weight much more than did the unfertilized trees; in the latter, the dry weight production kept constantly behind the increase in total potassium content. Phosphorus and nitrogen absorption were slower than the dry-weight increase. In the case of fertilized trees, during the July–September period, the dry-weight increase was far more rapid than was the absorption of potassium, or any other element. In the same trees during the period September 17–November 17, the total phosphorus decreased greatly. Yet in the unfertilized trees, phosphorus increased right to the end of the season (November 17).

In the early part of summer, nutrient absorption by these young trees was always far ahead of the dry-weight increase. It is of interest to note that BAUER stated that the *root respiration* of beech, ash, elm, maple, and larch trees in the period March 15 to May 24 was far ahead of the respiration of the tops in every case, thus explaining the great loss of dry weight the young tree undergoes in early spring, most of the loss being caused by root activity. Forest trees appear to have a higher iron content than most of our fruit trees, the roots especially being surprisingly high in this element. KÜBLER for beech trees reported ferric oxide equal to 5 per cent. of the ash in leaves; 4 to 9 per cent. of the ash in stems; and 7 to 13 per cent. of the ash in roots. In his *fertilized* trees, the iron content of *leaves* was about the same as in those just mentioned, but the *iron content of roots and stems was far lower* than was the case in *unfertilized trees.* The fertilizer

that was applied to the trees contained potassium as well as nitrogen and phosphorus. BAUER reported a similar iron content for various organs of fir trees, and a tremendous manganese content, equal to 22 per cent. of the ash in the old needles. KÜBLER, again working with beech trees, found that iron stored in the roots moved into the young shoots, and the root showed a great loss of total iron.

BAUER gives some interesting facts concerning translocation of elements within young (two or three-year-old) ash trees. On May 21, of the total potassium in the leaves, about 46 per cent. had come from old parts of the tree (root or stem), the remainder having been recently absorbed from the soil. Similarly 41 per cent. of the total nitrogen, 50 per cent. of the total magnesium, a small part of the silica, and *100 per cent. of the phosphorus* in these new leaves had come from storage in older tree parts. At this early period, enough calcium had come in from the soil to supply the needs of the leaves.

BAUER then presents his classification of the periodical activities of the ash tree as follows:

Period 1 (up to May 21).—This period is characterized by a using up of stored mineral and organic matter, with very slight absorption of soil nutrients.

Period 2 (May 21 to July 9).—Great leaf growth and slow root growth occur in this period.

Period 3 (July 9 to September 17).—The total-leaf dry weight decreases, and nitrogen, phosphorus, and potassium are lost from the leaves.

Period 4 (September 17 to November 17).—No great change takes place in either stem or root in spite of the backward movement of materials from the falling leaves.

In the new leaves (of the oak) formed in the spring, 40 per cent. of the nitrogen came from the soil direct. Later in summer, when the second-cycle shoot growth occurred, of the mineral elements moving into the young shoots, the old leaves (of the first cycle) furnished 24 per cent. of all the potassium, *100 per cent. of the calcium,* 52 per cent. of the magnesium, 26 per cent. of the phosphorus, and 62 per cent. of the nitrogen. The total iron content in old leaves decreased at this period, the new leaves obtaining some of their iron supply from the leaves of the first cycle of growth. Incidentally, the rate of mineral and nitrate absorption by oak trees appears to be rather slow throughout the season, as is also their general growth rate.

Finally, then, experiments on forest trees make it clear that here *the most rapid absorption for the season does not always occur at the time of maximum terminal growth of the top.* Where the young tree makes both first and second-cycle top growth, the maximum absorption (in per cent.

of the total for the year) is apt to fall either in the "resting" period between the two cycles of top growth, or in the early part of the second cycle. In young beech trees, in southern Germany, terminal growth is often over by June 1 or before. After a rest of three weeks or so, a second cycle of shoot growth begins. According to BAUER, maximum absorption of nitrogen and potassium in young beech trees occurred in July or early in August, in young ash trees in June, larch trees in August, etc. It is probable that periods of maximum root-length growth coincide with periods of maximum absorption of nitrogen and potassium. Phosphate absorption in forest trees is apt to occur either late in the season, or slowly and evenly throughout the summer and fall. Calcium absorption is usually greatest late in summer, although it is notable that calcium is rather constantly absorbed throughout the season by the broad-leaved forest trees, but not by conifers, apparently.

In rather early spring, calcium alone may be absorbed (as bicarbonate and chloride) by many trees. In the period prior to bursting of the buds, ash trees absorb a certain rather small amount of calcium, potassium, silica, and nitrogen, but no magnesium nor phosphorus. The presence or absence of large quantities of given elements in the soil solution at given times of the year does not usually play a deciding rôle in the time of maximum absorption of these elements by the tree. In fact RAMANN and BAUER suggested "mixed" type planting of woodlots to various tree species, so that utilization of nutrients, nitrogen especially, would be more efficient the year round. Thus fir trees may have completed absorption of nitrogen before the pine trees have even started absorption, etc.

On elemental starvation experiments with forest trees there has been comparatively little work, and most of it deals with conifer trees only. In Germany, VATER (**48**) and MÖLLER (**26**) starved a group of pine trees, etc., grown in pot cultures, for magnesium, sulphur, nitrogen, and phosphorus. BURGERSTEIN (**6**) found that his "minus-nitrogen" pine seedlings showed the usual symptoms of low-nitrogen plants, pale green leaves and stunting of growth, etc. Low-sulphur pines showed very small needles, delicate in texture and of pale yellow color. VATER found his low-phosphate pines grew needles of a bluer tinge than normal plants. MÖLLER found that phosphate or sulphate starvation reduced the development of his pine seedlings more than did magnesium starvation.

In the case of fruit trees, elemental starvation studies have been made recently by WALLACE. At an earlier period MÜLLER-THURGAU (**27**) grew young pear trees in low and high nitrogen, phosphorus, potassium, and calcium cultures, respectively, for a period of four years. REMY (**37**) also grew apples and pears in low nitrogen, potassium, phosphorus, and calcium over a three-year period.

WAGNER (**52**) reported on 20 years' work in fertilization of trees grown in soil, reporting on pears in low nitrogen, phosphorus, and potassium, as well as on complete fertilizer trials. WAGNER found that after a long period of *potash* starvation, his pear trees gave very poor quality fruit. By other starvation treatments, apparently the quality of the fruit was not greatly affected. Low potassium did not give him an increase in percentage of nitrogen in the leaves, as has been elsewhere reported. Low-nitrogen leaves were very high in phosphate, and contained only 1.23 per cent. nitrogen on a dry-weight basis. The low-phosphate leaves were highest of all in nitrogen (1.69 per cent. dry weight). The complete-fertilizer leaves had 1.5 per cent. nitrogen. The low-phosphate leaves were highest of all in calcium.

REMY found that low nitrogen (under 1.25 per cent. of dry weight) in apple and pear leaves during ripening of the fruit was indicative of insufficient nitrogen for full blossoming in the following year. REMY (**38**) seems to have grown apple trees in water cultures, starving the trees for phosphorus, in order to determine which organs of the tree showed the effects of low phosphate most markedly. He found that the young vegetative growing points were *not* easily affected by phosphate starvation, but that the older leaves and stem parts were most readily brought to low phosphorus content in the ash.

MÜLLER-THURGAU (**27**), in his work with pear trees grown for four years in low-potassium cultures, found chlorosis appearing only in the last two years. In his low-nitrogen trees, in the third year only a slight chlorosis appeared; otherwise apparently no leaf troubles were brought on by the treatments. For the entire four years, the poorest yield of fruit was in the lot of low-potassium pear trees. The highest yield came from high-nitrogen trees, followed by high-calcium, high-potassium, and low-phosphate trees. Apparently his soil was well supplied with phosphorus. Nutritional conditions giving high fruit yields did not necessarily seem to give large trunk circumference increases. By the latter measure of growth, low-calcium trees gave the greatest increase, although the fruit yield of the trees was poor. In the fourth year of starvation, the low-potassium trees yielded only one-thirtieth as much as the high-potassium trees.

STEGLICH (**43**) made ash analyses of entire trees, fruit and all, of apple, pear, plum, and cherry. The series of researches, of which STEGLICH'S work was a part, covered a period of 13 years. STEGLICH stated that the yearly nutritional needs for the total growth of one "average" fruit tree in moderate bearing, and 25 cm. in circumference were as tabulated on page 10.

Similar studies were made at a later date by VAN SLYKE, TAYLOR, and ANDREWS (**47**). STEGLICH also calculated the rate at which the yield of

Variety	N	P_2O_5	K_2O	CaO
	gm.	*gm.*	*gm.*	*gm.*
1. Apple tree	59	11	51	109
2. Pear tree	37	7	40	69
3. Cherry tree	76	30	95	209
4. Plum tree	34	11	74	75

fruit increased in various species of fruit trees, per cm. increase in trunk circumference, also the total weight of foliage of one tree per cm. circumference. He found that in case of cherry trees, foliage weight was equal to 358 gm. per cm. trunk circumference, while with pear trees the foliage weight was only 105 gm. on the same basis, with apple and plum foliage ranking somewhat above pear foliage. Conduction and transport to the leaves in the cherry must be a more serious problem than in the case of the pear tree. Cherry trees also must be classed as heavy feeders on most of the nutrient salts of the soil.

Varin Simon had calculated that a fair sized fruit tree, spreading over 20 square meters of land, produced per year 15 kilos of leaves, 8 kilos of wood, and 100 kilos of fruit. Degenkolb, Barth, and Steglich (**9**) compiled average ash analyses of fruit trees from all over Germany. Of apple, plum, cherry, and pear, the plum leaves ran far higher in nitrogen, phosphorus, and potassium on percentage dry weight than did leaves of the other species.

Wagner (**52**) reported on 20 years' work in fertilization of trees grown potassium, magnesium, calcium, and phosphorus respectively, over the rather long period of three years. In his last experiment (1929) he used eighty apple trees on Malling Type Ten standard vegetatively propagated stocks, with twenty trees in each starvation group. Part of his trees were summer-pruned. The leaves and prunings were collected in July, analyzed each year, and the entire trees analyzed at the conclusion of the three-year experiment.

It is well to note that Wallace's complete culture solution added to the sand was high in K/N ratio, but was otherwise much like the usual type of field-crop culture solution. So little is known of the needs of trees in the way of mineral nutrition that no particular culture solution has ever been worked out for tree cultures. Wallace reported that growth of the check trees was satisfactory, except for brown spots on the leaves caused by excessively high potassium concentration in the culture. The final results of the three years of starvation did not show entire agreement with his previous results from similar starvation experiments with another variety of apple tree. Usually a minus-potassium treatment results in greatly de-

creased shoot growth, but in WALLACE's late experiments no great checking of terminal growth was observed in such trees. Also, minus-calcium trees gave better shoot growth than did complete-culture trees. But in minus-magnesium cultures, shoot growth was greatly reduced; and by the third year the leaves of these trees were chlorotic, thin in texture, and showed severe breakdown or browning of the tissues. More or less breakdown occurred on leaves of all of his cultures during the later years of the experiment.

Root growth in WALLACE's minus-calcium trees was again reported as being very good. Previously he had reported that low-potassium, low-magnesium, and low-phosphorus trees all produced very poor root systems, more or less injured and blackened. These trees (Cox's Orange Pippin) were also grown in sand cultures. WALLACE believed that his low-potassium tree root systems were too small to absorb water enough for the needs of the top, and that leaf scorch resulted as a consequence.

In his latest work, WALLACE (**49**) has shown how difficult it is to reduce the potassium, magnesium, or calcium content of leaf or bark tissue in trees by starving them for these particular elements. *After three years* in a minus-potassium culture, leaves of the trees concerned had an ash containing 10.6 per cent. K_2O on July 7 of the last season, when 20 per cent. K_2O would perhaps have been ample for normal foliage. His minus-calcium leaves of the same date still had 70 per cent. of normal calcium content; and minus-magnesium leaves still had 40 per cent. of normal magnesium content.

In the summer-pruned series, stems and petioles (prunings of July 7 of *third* year of starvation) showed for the minus-calcium series CaO equal to 94 per cent. of the normal CaO content; the minus-potassium series showed 55 per cent. of normal MgO content.

Concerning the interrelated effects of elemental starvation, minus-potassium tissue was usually high in phosphorus. Calcium starvation did not appreciably raise the potassium content. The potassium content of the complete-culture trees was so high in this case that probably no treatment could have raised it materially without death to the tree. Unfortunately, WALLACE does not include the nitrogen content of his trees in the analyses recorded. As to the total ash content of starved tissue, only magnesium starvation raised the percentage of ash (on a dry-weight basis) in leaves, bark, and wood above the normal content. Potassium or calcium starvation resulted in a lower ash content throughout the tree. Also, the absolute percentage of ash seemed to fluctuate from year to year, within the various groups, just as it does in grain crops, etc.

There are certain things in WALLACE's work that will bear questioning. WALLACE himself states that apparently his calcium-starvation treatment

was not 100 per cent. effective in cutting off the supply of calcium to these trees. The sand used as a culture medium was 99.5 per cent. insoluble in hydrochloric acid. Yet obviously the trees obtained a fairly adequate supply of calcium from the small percentage present as impurity in the sand. Each tree had access to ten liters of sand in the pot. And for three years these trees, though slightly reduced in calcium content, produced normal growth, or better than that of the check trees. No leaf mottling nor chlorosis was reported. It is a curious fact that trees (and annual plants), if partially starved for calcium, fill up their tissues, especially their leaves, with silica. In a sense then, in sand cultures, silica may act as a sparer of calcium in the formation of the middle lamella and elsewhere.

Some time ago, REED and HAAS found that roots of citrus trees, grown in water cultures, were extremely sensitive to the absence of calcium in solution. These roots were able to grow for long periods in a simple calcium chloride solution, with all other elements lacking. They did not grow well in distilled water, unless calcium was added. This may be explained in part by the presence of traces of copper in the solution, but none the less, calcium plays a unique rôle in keeping these roots alive and growing. The growing point is particularly sensitive to the absence of calcium in the culture solution.

After WALLACE's earlier papers, it was thought that perhaps deciduous fruit trees were not at all sensitive to the lack of calcium, and were consequently very different in nutritional requirements from the species of citrus trees used by REED in his experiments. It must be recalled that REED worked with water cultures, while WALLACE's work was with sand cultures; the results are therefore not entirely comparable, the sand furnishing both calcium and silica, and affording better aeration for the roots.

Which type of culture more closely approximates soil conditions? A fine sandy loam soil may well be compared with sand cultures, but a heavy and more or less wet clay loam soil is more likely to provide conditions closely resembling, in many respects, an aerated water culture. Yet in either case there should be little doubt that calcium is necessary for the growth of trees, as well as for all other plants, except possibly the lowest algae and certain fungi. The actual amount of calcium needed by some species of fruit trees may be small.

Sand cultures are very apt to contain fair supplies of calcium and iron, but much less likely to hold adequate supplies of potassium, nitrogen, etc. Consequently potassium and nitrogen-starvation experiments may be carried out in sand cultures to much better advantage than can calcium-starvation tests, as WALLACE's work very clearly shows.

Experimentation

The purpose of this work was to study the course of potassium and nitrate absorption, and the general nutrition of young two and three-year-old French prune trees throughout the season. These trees were grown in water culture during the course of the experiment; and the culture solution itself was studied in determining the amount and time of absorption of various elements. The water-culture method was used because it allows a comparatively easy and accurate study of absorption, when only a small number of trees is used; also, the identical trees are used throughout the entire season. The alternate method of absorption study is to harvest a certain number of entire trees (roots and tops) periodically throughout the season, analyzing the ash of the whole tree each time. A great many trees must be used in such work, at least 100 trees at each date selected for study; and the time and labor required for digging tree roots from the soil, careful washing, drying, grinding, weighing, ashing the tissues, and analyzing the ash is excessive.

The work was begun in 1928, and carried through the summer of 1930. Some of the trees were grown for three years, others for only two years in water cultures in greenhouses at Berkeley. French prune trees were used throughout the study, with a few apple and pear trees for general observation.

In April, 1928, and March, 1929, one-year-old whips budded on Myrobalan stocks were pruned to a 30-inch head, the roots washed carefully, the entire tree weighed, and set up in jars of aerated culture solution in the greenhouse. The jars used were 4-gallon crocks painted on the inside with black asphaltum. Jar covers were gypsum casts, about one inch thick, the covers being boiled in high-melting-point paraffin and painted with asphaltum. The trees were held in place by fairly loose-fitting corks. Continual aeration was supplied through glass tubes to the bottom center of the jars, which were kept about two-thirds full of solution. The jars were well packed in damp moss on tables about three feet from the ground. The greenhouse temperature was lowered during the day by an electric fan at each end of the greenhouse. Air temperature at the level of the tree foliage was recorded daily at about 11 A. M.

Culture solutions were changed every three or four weeks, or more often if a low-nitrate level made it necessary. Constant checks were kept on the supply of nitrate in the cultures by means of diphenylamine tests. The modified HOAGLAND'S solution used for complete cultures contained the following ion concentrations: $NO_3 = 700$ p.p.m.; $PO_4 = 10$ p.p.m.; $SO_4 = 147$ p.p.m.; $K = 150$ p.p.m.; $Mg = 37$ p.p.m.; $Ca = 150$ p.p.m. Total = 1194 p.p.m.

Foliage color was kept good to excellent in the complete solution cultures by the following iron treatment. Particularly in the early part of

each season, iron was added to each jar frequently, the salts used being both ferric chloride and ferric tartrate. In addition, HOAGLAND'S A-Z mixture was used, the mixture introducing nine other cations and three other anions to the stock solution.

HOPKINS (**13**) recently found that increasing the amount of citrate in the solution depressed the iron-ion concentration, and that the growth curve of the green alga, *Chlorella,* closely followed the changing concentration of iron ion in the surrounding medium. Within limits, the higher the iron-ion concentration, the greater was the growth of the alga. HOPKINS apparently holds the view that only iron ion is absorbed by plants; more soluble iron would not be available to the plant, unless in the ionized state.

The group of trees intended for starvation included 85 one-year-old prune trees, from which number five were immediately taken out for ash analysis, to serve as a check on the initial condition of the trees, with special reference to the storage of mineral elements and nitrogen. The remaining trees were all set up in the greenhouse, ten trees in each of the groups, as shown in table I. (Average fresh weights are given in each case, for March, 1929, the beginning of the experiment; for December, 1929; and the final weights in September, 1930.)

TABLE I

FRESH WEIGHT INCREASES MADE BY YOUNG FRENCH TREES (STARVATION SERIES)

TREATMENT: CULTURE SOLUTION	AVERAGE INITIAL WEIGHT MARCH, 1929	AVERAGE FRESH WEIGHT DEC., 1929	GAIN	AVERAGE FRESH WEIGHT SEPT., 1930	GAIN (TOTAL)
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Complete	123.0	528.0	405.0	1072.0	949.0
–K	109.4	265.4	156.0	316.0	207.3
–K + Na	132.5	307.5	175.0	386.7	254.2
–Ca*	121.4	206.4	85.0	249.0	127.6
–N	129.8	259.8	130.0	303.0	173.2
–Mg	116.0	294.0	178.0	338.7	222.7
–P	120.2	394.2	274.0	421.0	300.8
–S	120.5	461.5	341.0	673.5	553.0

* –Ca = actually low Ca solution.

The average initial fresh weight of the entire group was 122 gm. The maximum deviation of a single group from the general mean was approximately 10 per cent.

Also, data from French prune trees grown in the greenhouse the previous year (1928), as well as in 1929, are shown in table II.

TABLE II

FRESH WEIGHT INCREASES MADE BY FRENCH PRUNE TREES OVER A TWO-YEAR PERIOD WITH VARIOUS CULTURE TREATMENTS

GROUP TREATMENT	AVERAGE INITIAL WEIGHT APRIL, 1928	FINAL FRESH WEIGHT DEC., 1929	GAIN
	gm.	*gm.*	*gm.*
Ten trees in complete nutrient solution	178.0	946.0	768.0
Ten trees in –K	198.0	434.0	236.0
Five trees in distilled water	164.0	296.0	132.0

General growth behavior of the trees

It is apparent from table I that the group of trees in minus-calcium solutions made the poorest growth, followed in turn by the trees in minus-nitrate, minus-potassium, minus-magnesium, minus-phosphate, and minus-sulphate solutions. The last-named trees made nearly normal growth, particularly as regards root development; and their total nitrate absorption during the season (1929) was practically equal to that of complete culture trees. On the other hand, minus-calcium trees failed to produce any roots until calcium was added to these cultures for a period of about six weeks, after which they were returned to a minus-calcium solution. The roots of French prune (Myrobalan stock) refused to grow in water culture unless at least a trace of calcium was present in solution. The same situation was found in the case of apple roots (crab stocks), pear roots (French stocks), and peach or almond seedling root stocks. The latter two species were particularly sensitive to a lack of calcium in solution. These facts may appear contradictory to WALLACE's findings for apple roots, but the explanation regarding the sand cultures used by WALLACE has been mentioned.

Figure 1 shows the comparative fresh-weight increase, shoot-length growth, and diameter increase of one-year-old French prune trees during the season of 1929; and figure 2 gives the fresh-weight increase, shoot-length growth, and diameter increase of two-year-old trees during the second year of treatment.

MEVIUS's (**24, 25**) work on minus-calcium cultures and root growth of various annual plants, as well as the recent work of KOSTYTSCHEW and BERG (**19**) on the form of calcium present in living tissue, involving the supposition that part of the active calcium is adsorbed by plasma colloids, is full of interest in this regard. Also, WARTHIADI (**53**), working with wheat plants, found that sand cultures behaved somewhat differently than did water cultures, in which, if the Ca/Mg ratio was 1/10 or 1/20, all the wheat

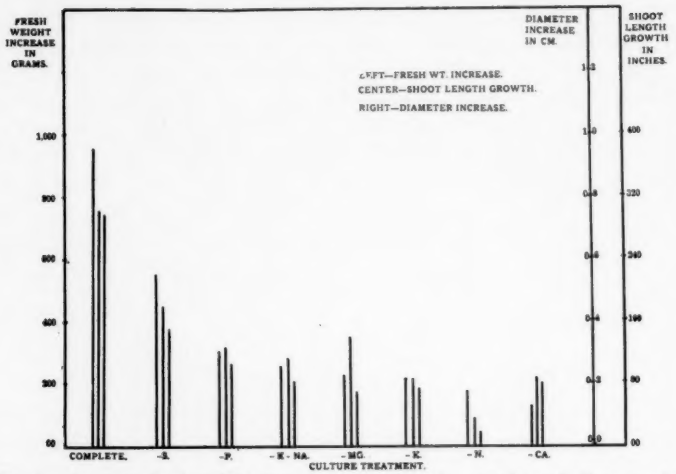

FIG. 1. Average fresh-weight increase, shoot-length growth, and diameter increase per tree. One-year-old French prune trees, season of 1929.

plants died early. In sand cultures, the 1/20 Ca/Mg ratio plants lived, but formed heads containing only chaff. No tillers formed heads in this case. The plants in 1/1 and in 20/1 cultures matured normal heads, in both sand and water cultures.

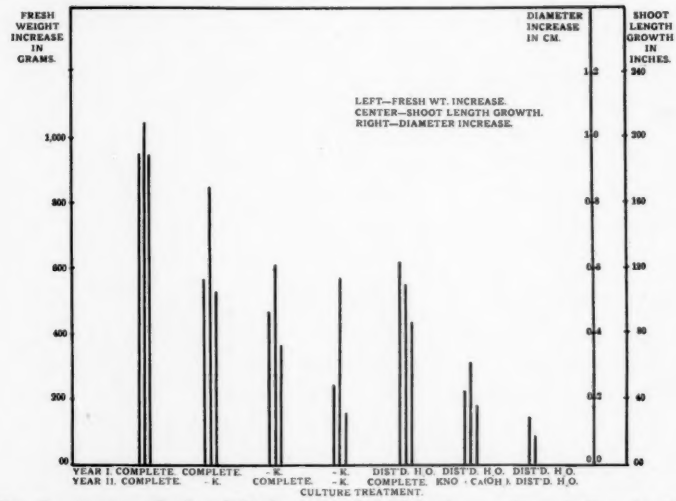

FIG. 2. Average fresh-weight increase, shoot-length growth, and diameter increase made by two-year-old French prune trees in the second year of treatment (1929).

In regard to fruit trees, the white growing roots of French prune trees when placed in a minus-calcium solution soon turned brown or black, and finally died back to the main root. Calcium did not appear to migrate easily in these roots. Portions of a single root system immersed in a plus-calcium solution did not enable the remainder of the root to make a normal growth. The shoot growth of the low-calcium trees was very poor in both years of the experiment. A slight mottling of the leaves on some of the trees was noticeable the second year; otherwise foliage color was good. Each season, however, the foliage was lost rather early (usually in August), the leaves wilting and drying on the tree, apparently suffering from a lack of water.

TABLE III

GROWTH AND ABSORPTION OF ONE AND TWO-YEAR-OLD FRENCH PRUNE TREES IN WATER CULTURE

TREATMENT	FRESH WEIGHT INCREASE	DIAMETER INCREASE†	TOTAL NO_3 ABS'D* IN 1929	TOTAL PO_4 ABS'D IN 1929
GROUP 1.	*gm.*	*cm.*	*gm.*	*gm.*
Two-year period 1928–1929 — Two years in complete solution	765.2	0.96	24.7	0.52
Two-year period 1928–1929 — Two years in –K solution	235.6	0.155	6.0	0.31
First year in –K; second in complete solution	462.0	0.350	14.4	0.38
First year in complete; second in –K	572.8	0.530	17.5	0.45
First year in distilled H_2O; second in complete	616.0	0.430	21.7	0.33
Two years in H_2O	132.8	0.07	.....	.....
GROUP 2. One-year period — One year in –K solution	156.3	0.110	9.975	0.345
One year in –K + Na solution	177.3	0.147	8.376	0.358
One year in –Ca	85.0	0.062	2.354	0.178
One year in $-NO_3$	130.1	0.028	.....	0.190
One year in –Mg	177.5	0.148	7.805	0.275
One year in $-PO_4$	274.4	0.149	7.168	.....
One year in $-SO_4$	341.5	0.215	15.808	0.365
One year in complete	405.5	0.324	20.810	0.480

* Nitrate and phosphate absorption were measured only during 1929.

† Diameter increase is caliper increase of trunk, 3 inches above the bud.

The minus-potassium trees grew moderately well the first year. The second year both shoot and root growth were much reduced, although many white roots were still produced. Potassium-starved roots were in far better condition throughout the two years than were the calcium-starved roots. Leaf scorch and chlorosis appeared in the late summer of the first year, and severe scorch again early in the second summer. During the second season, liberal supplies of iron prevented all but a trace of chlorosis, but did not prevent leaf scorch. It seems that leaves can be kept green, if plants are either in a high-potassium + low-iron solution, or in a low-potassium + high-iron solution. However, after three years of severe potassium starvation, the trees were chlorotic in spite of an abundant iron supply (both injection and solution supply). The diameter growth of the trunks of the minus-potassium trees was greatly checked as the growth tables show (table III). Minus-potassium + sodium trees showed conditions similar to the preceding in every respect. The presence of sodium did not have any effect in preventing leaf scorch or chlorosis.

WIESSMANN (**54**), working on barley plants, found that –K + low N plants matured grain and were nearly normal, while –K + high N plants never formed any heads, and produced only two-thirds as great a dry weight as the –K + low N plants. Absolute potassium starvation, then, must be studied in the light of relationships with nitrogen supply levels, and probably with other elements as well.

JAMES (**15**) has reported on work concerning the physiological rôle of potassium in annual plants. He showed that the amount of starch formed per unit leaf area increased with an increase in potassium content. Potassium itself tended to decrease leaf size (unlike calcium), but potassium chloride increased leaf size, the chlorides having the power to increase water content and size of leaves. The apparent rôle of KCl, in increasing translocation of carbohydrates from the leaves, may possibly be explained on the same basis; *i.e.*, the greater water supply circulating to and from the leaves, caused by the presence of chlorides. Potassium sulphate did not appear to increase translocation of carbohydrates from the leaves.

Trees starved for nitrate showed the usual symptoms of low-nitrogen orchard trees: most severely stunted twig growth, pale yellow-green leaves, a reddish brown bark, and a thin, long, stringy type of root system. The roots continued in active growth throughout most of the entire two years, but terminal top growth lasted only a few days.

The magnesium-starved trees produced very good root growth throughout the two years. The shoot growth was fair the first year, but greatly stunted during the second year, when virtually complete defoliation occurred before July 1 (beginning in May). Severe marginal browning of the leaves, gradually extending back to the midrib, occurred early in both

summers. The symptoms of low-magnesium leaf injury are very typical, the injured portions assuming a dark chocolate color, preceded by olive-green colored areas, water-soaked in appearance. Only a trace of low-magnesium chlorosis appeared the second year, when the iron supply was kept at a high level.

The low-phosphate trees at first made splendid root growth, but later growth was greatly reduced, and very little new root growth was produced during the second year. All roots in the cultures of low phosphate showed darkened tips. Fair shoot growth was made by these trees but was not comparable with normal tree growth; defoliation was exceptionally early in both summers, the earliest of any of the starvation series. In the second summer the leaves were about one-half normal size, and all of them bronzed or turned yellow and abscissed in June. The trees made no more new growth after this defoliation. Low-magnesium trees, on the other hand, continually opened up new buds, making feeble shoot growth, only to have that in turn wither and defoliate.

BUTKEWITSCH (**7**) found that low-phosphate oat plants grew better at pH 5.5 than they did at pH 8.0. The minus-phosphate solution used in the present experiment had a pH of 6.0–6.6. He also notes that low-potassium plants grew better at pH 8.0, quite the opposite situation to that of low-phosphate cultures.

As regards the bronzing (or purpling) of leaves or plants grown in low-phosphate solution, it is suggested that the unusual color development may be the indirect effect of the increased solubility and total supply of iron in the leaf and other active tissues of low-phosphate plants. Soluble-iron compounds are capable of reacting with phenolic, or tannin-like substances, to give various color developments,—brown, purple, red, etc.

The sulphur-starved trees showed excellent root growth in both years, and fair to good shoot growth. Very little premature defoliation occurred in this series, although both brown leaf spots and a pale, light yellow, non-veined chlorosis appeared in both years. Although this sulphur-induced chlorosis is very different in appearance from that produced by low potassium, or by low magnesium, yet in all three cases the development of the typical chlorosis is greatly postponed by high iron supply.

A type of little-leaf can be produced by sulphur starvation treatment, especially if iron is at the same time not above normal. BURGERSTEIN and MÖLLER noted that very small, pale colored needles were produced on young pine trees grown in minus-sulphur cultures. The small leaves produced by low-sulphur French prune trees were usually irregular and lobe-shaped as well as diminutive in size.

The complete-solution trees made good root and shoot growth in both years, and carried well colored foliage. However, the total terminal and

diameter growth was not equal to that produced by well-cared-for orchard trees of the same age in fertile soil in California.

Seasonal absorption of nitrate and potassium

During 1929, from May to November, the nitrate absorption of trees of the complete-solution series, as well as those of the various starved groups, was followed, samples being taken from the culture solution every two weeks for nitrate analysis, and for conductivity readings. Nitrate concentration was determined colorimetrically by the phenoldisulphonic acid method. At nitrate levels of 200–300 p.p.m., the error of the method is about 5 per cent., but at low levels of nitrate the error is often more than 10 per cent. The initial level of nitrate in the solutions worked with was 700 p.p.m., and analyses showing levels as low as 5 p.p.m. were regarded as equal to zero, the solution being discarded after checking with diphenylamine and phenoldisulphonic acid. Conductivity measurements were regarded as indicating approximately the course of total salt absorption throughout the season, although it is true that nitrate absorption is revealed by the method, almost to the total exclusion of phosphate absorption, etc.,

TABLE IV

Seasonal absorption (by periods) of nitrate by one-year-old French prune trees (ten trees per group); NO_3 expressed in grams

No.	Season	Solutions used						
		Complete*	−K	−K+Na	−Ca	−Mg	$-PO_4$	$-SO_4$
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1.	May 17–June 1	3.17	2.856	2.154	No	1.484	−0.336	2.527
2.	June 1–June 15	0.07	1.365	1.656	Root	2.044	3.920	1.575
3.	June 15–June 29	3.22	1.057	0.941	Growth	0.896	0.210	2.191
4.	June 29–July 29†	5.28	4.326	3.585	0.67	3.857	4.109	5.229
5.	July 29–Aug. 10	0.33	0.483	0.466	0.03	0.693	0.364	0.147
6.	Aug. 10–Aug. 24	0.38	−0.238‡	0.163	0.68	0.07	−1.008	−1.225
7.	Aug. 24–Sept. 6	0.91	0.021	−0.350	0.17	−0.217	0.728	1.274
8.	Sept. 6–Sept. 20	0.89	−0.714	−0.753	0.31	−1.309	−0.819	0.672
9.	Sept. 20–Oct. 5	0.91	0.798	0.552	−0.11	−0.310	−0.728	1.316
10.	Oct. 5–Oct. 19	1.05	0.826	0.428	0.60	0.366	0.042	0.819
11.	Oct. 19–Nov. 2	1.80	−0.665	−0.466	.....	0.371	0.686	1.281
12.	Nov. 2–Nov. 16	0.28	.....	.....	.....	.....	.....	.....
	Total	18.29	9.975	8.376	2.35	7.805	7.168	15.808

* The complete group here included 15 trees.

† Period of four and one-half weeks.

‡ Minus signs indicate NO_3 lost from the trees back to the culture solution.

and that base exchange and bicarbonate exchange all make conductivity measurements of absorption only approximate.

As has been stated, daily temperature was recorded in the greenhouse throughout both summers (1929 and 1930). A fair correlation between temperature and nitrate absorption (fig. 3) seems to exist. A period of unusually high temperature (with eight consecutive days above 90° F.) and low humidity came at the end of June of the first summer, and the maximum rate of nitrate absorption for the season occurred in this same period. Aside from high temperature, the intensity of sunlight probably reached a maximum at this period also, although no records were kept of it. Top and root growth was fairly active in this period also.

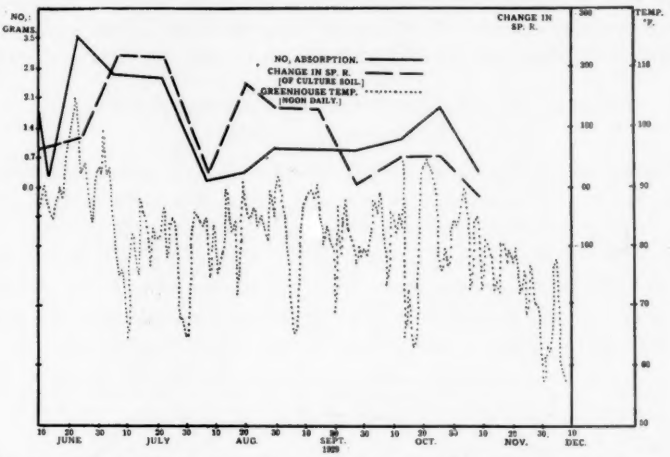

FIG. 3. Rate of nitrate absorption by one-year-old French prune trees in complete culture solution, and the changes in the specific resistance of the culture solutions, plotted with the greenhouse temperature, recorded daily at 11 A. M. Absorption data based on average of 15 trees, during season of 1929. Grams of nitrate per tree per two-week period.

The next high temperature period (fig. 3) fell later in the season, about October 22, with four days of heat above 90° F. At this time root growth and trunk-diameter growth were proceeding rather rapidly, but terminal growth had stopped long before. The second peak in nitrate absorption for the season fell in this period, but was quickly followed by an abrupt decline, as the roots soon went into winter dormancy. Then very slow root growth, or none at all in some cases, was made up to the last days of January, 1930, when vigorous new white feeding roots again appeared, long before the buds started to swell. The trees were placed outdoors in winter.

Between the two peaks of the nitrate absorption curve lay a brief period of very slow absorption, accompanied by low air temperature, followed by a long period characterized by a very uniform rate of nitrate absorption. Long continued periods with temperatures (daytime) below 85° F., probably accompanied by cloudy weather, seemed to be very unfavorable for nitrate absorption in case of the French prune trees. However, very slow nitrate absorption did take place in most of these trees through December, when no temperature data went above 80° F.

The absorption curve presented for 1929 began about June 1. Considerable nitrate absorption occurred in these trees in May, and less in April, as shown by the curve for the same trees for the season of 1930, but the nitrate intake in May was usually below the peak reached in late June. The conductivity curves showed a maximum rate of total absorption in the period July 10–July 30, slightly later than the peak in nitrate absorption.

Seasonal nitrate absorption by two-year-old French prune trees in 1930

The nitrate absorption of these same trees during their second year of growth in culture solutions was followed, from March 20 to December 1 (table V). Potassium absorption was also followed with the same trees during 1930 (fig. 4). Again it was found that there was a high correlation between daily (noon) temperature in the greenhouse and rate of absorption of both nitrate and potassium. The only marked exception to the correlation came about May 1, midway in the period of rapid terminal shoot

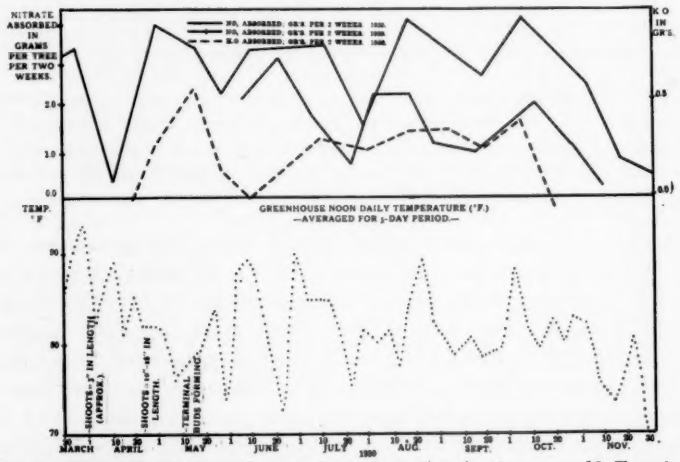

Fig. 4. Rate of nitrate and potassium absorption by two-year-old French prune trees growing in complete culture solution. Average of ten trees in each group.

growth. At this point nitrate and potassium absorption was rapid, in spite of rather low average temperatures. Rapid terminal shoot growth seemed to be an important factor in the rate of nitrate and potassium absorption in these trees. During most of the season, however, temperature appeared to play a dominant rôle.

TABLE V

NITRATE AND POTASSIUM ABSORPTION BY TWO-YEAR-OLD FRENCH PRUNE TREES IN COMPLETE SOLUTION (GRAMS PER TREE); 1930

No.	PERIOD	NO_3 ABSORBED	K_2O ABSORBED
		gm.	*gm.*
1	March 15–April 1	3.185	.....
2	April 1–April 21*	0.623	–0.044
3	April 21–May 5	3.710	0.240
4	May 5–May 10	3.360	1.080
5	May 19–June 2	2.080	0.280
6	June 2–June 16	3.020	–0.040
7	June 16–July 2	3.560	0.260
8	July 2–July 16	3.180	0.600
9	July 16–August 11*	3.090	0.800
10	Aug. 11–August 25	3.780	0.720
11	Aug. 25–Sept. 8	3.248	0.700
12	Sept. 8–Sept. 29*	3.598	0.780
13	Sept. 29–Oct. 13	3.696	0.840
14	Oct. 13–Oct. 27	2,891	–0.140
15	Oct. 27–Nov. 10	2.303	.....
16	Nov. 10–Nov. 24	0.644	.....
17	Nov. 24–Dec. 16*	0.665	.....
	Total	46.9†	5.820

* Period longer than two weeks.

† N equivalent = 10.5 gm. of total NO_3.

It may be noted (fig. 4) that the nitrate-absorption curves of two-year trees for 1929 and 1930 agree as to the dates of maximum absorption. The curve for the one-year trees (1929) showed two of the maximum points in common with the curves of absorption by older trees, but showed depressed absorption early in August, in contrast with rapid absorption going on at that time in the case of older trees. One-year trees can hardly be considered typical of usual tree growth or absorption in the orchard, and certainly not in the greenhouse. Trees newly placed in water cultures are late in starting growth, and seldom show second-cycle growth in late summer.

The rate of potassium absorption, it may be noted, usually lags slightly behind that of nitrate absorption, although the rate falls off more abruptly at the end of the season (November 1). Actual exosmosis of K from the

root back to the culture solution takes place at that time. The final drop in the nitrate and potassium absorption curves was correlated closely with leaf fall.

Although regular temperature records were kept only of the air temperature of the greenhouse, some readings taken of the temperature of the culture solution in the jars indicated that the temperature of the solution was being held rather uniformly at low levels, below 60° F., even when the air temperature at mid-day reached 90° F. The jars were well packed in damp moss and were of sufficient capacity to resist marked temperature changes. Any changes of temperature that did occur in the solution of course followed the same trend as that of the air temperature.

Many investigators, dealing with annual plants, have studied the effects of temperature, light, and humidity on the rates of salt absorption. Usually the conclusion has been drawn, from short duration experiments, that humidity and transpiration have no effect on salt absorption other than to increase the percentage of silica or chlorine in the ash of the plants exposed to high transpiration. Some exceptions to this view have been reported. Regarding the effects of light intensity, WIESSMANN (**55**) reports for rye, barley, and wheat grown in sunlight and in shade, that the former showed the higher total absorption of nitrogen, phosphorus, and potassium. The work of SEIDEN (**42**) is interesting. Maximum total salt absorption by his plants occurred in the afternoon of each day, at the time of greatest light intensity and highest temperature. Also the percentage of total ash on dry-weight basis increased with rise in temperature of the environment in which the plants were grown. The color of the light to which the plants were exposed also affected the ash content.

Recently PETRIE (**30**) investigated the effects of temperature on the unequal intake of ions of single-salt solutions by plant tissue. Instead of using entire growing plants, only portions (thin disks) of carrot root were used for the experiments. Such results as he obtained are only indirectly applicable to the problem of salt absorption by entire growing plants, but they appear to be of value, none the less. PETRIE found that at higher temperatures (20° C.) the absorption of cations of single salts is decreased, and the absorption of anions correspondingly increased. The product of the residue of anion and cation remaining in the outer solution remained a constant regardless of temperature. At low temperatures (4° C.) the reverse situation held true (cations being absorbed in excess). Equal rates of absorption for both cations and anions occurred at about 15° C. PETRIE pictures the ions (after absorption) as being held, adsorbed on the surfaces of the negatively charged colloidal particles of protoplasm in the cells of the plant tissue. He proposes that the same process is the basis for selective salt absorption by entire growing plants. Perhaps it is not surprising,

then, that growing fruit trees should show increasing rates of nitrate absorption at higher temperatures.

With forest trees, BAUER and KÜBLER have shown that throughout most of the season, dry-weight production lags well behind nutrient salt absorption, when both are expressed in percentages of the total increase for the entire season. Presumably the same situation holds in fruit trees. Terminal shoot growth may be active in early spring, but the total dry weight of the tree decreases instead of increases at this period. Later in summer, however, there is with forest trees a fair correlation between dry-weight increase and the rate of salt absorption by the roots. It seems fairly certain that nitrate absorption occurs only in growing roots, or at least in white roots, but not in suberized roots. It is doubtful whether any salt absorption (with exception of chlorides) can take place through suberized roots. Experience in the rooting of peach and apple trees seemed to indicate that chloride absorption may occur through old suberized roots in these trees. Leaves and young shoots were forced out much more rapidly on trees with roots in chloride-containing solutions than on trees not receiving chlorides. None of the other salts seemed to affect growth in this way. It should be noted, of course, that dormant trees placed in water solutions always form shoots before any new white absorbing rootlets are formed.

BAUER has discussed the question as to whether or not old fruiting forest trees possess the same or similar seasonal absorption characteristics as the young forest trees that were used in his studies in absorption. He believes that age would make very little difference in the matter, although the oldest trees were four- or five-year-old seedlings. It seems possible that with fruit trees (apple or pear), a heavy crop of fruit might increase the demand for, and absorption of, most nutrients late in the season. At present we have no evidence on the question. Late summer nitrating of apple or pear orchards is usually undesirable, from the standpoints of both fruit color and winter hardiness.

In the case of the French prune, so far as the maximum rate of nitrate absorption is concerned, nitrate fertilization is apparently most needed in late June and early July. The value of nitrate fertilization early in spring, and its effects on fruit setting or on fruit-bud formation, are questions outside our present discussion. A relatively small amount of nitrate added at the right time may be more important in determining the size of the yield than larger amounts of nitrate applied at other times of the season.

It may be noted here that probably not all fruit trees have their maximum nitrate absorption at the same period of the year. Nitrate absorption curves for a few Delicious apple trees (grown in water culture also) showed in early summer a slowly rising nitrate absorption with a small peak about June 25, corresponding to the nitrate maximum rate in the French prune;

but the second peak, the maximum rate of absorption for the whole season, fell on September 2, and the final smaller peak on November 10. The leaves of these trees were green until December. Second-cycle terminal shoots were still growing on September 2.

Nitrate absorption by starved trees (one year old)

As has been stated, the seasonal absorption of nitrate by the French prune trees was followed through 1929, for the whole series of starved trees, as well as for those trees in complete cultures. The amount of nitrate and phosphate absorbed for the season was determined for trees of each starvation group, as well as the tree-weight increase, the diameter increase, the total length of shoot growth, etc., during the year of the study.

The set of curves of total nitrate absorption of trees starved for various elements presents a rather interesting situation (figs. 5, 6). Trees starved

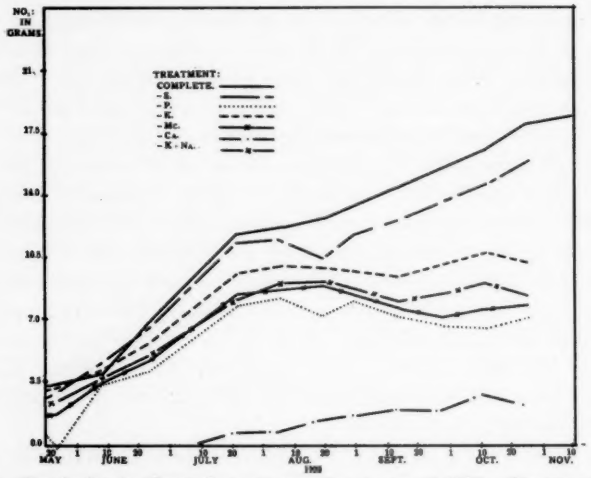

Fig. 5. Total nitrate absorption per tree for season of 1929. One-year-old French prune trees in various solutions; ten trees per group.

for sulphur throughout the year show both a rate and total amount of nitrate absorption very nearly equal to that of complete-solution trees. In fact, early in July the –S trees showed a higher rate of nitrate intake than did the complete, or any other treatment group of trees. This fact might be anticipated, if we consider that SO_4 is a competitor of NO_3 in total salt absorption, and if we assume that the –S trees are not very greatly depleted in sulphur at this early time in the season, an assumption which is probably true, since the tree growth was nearly normal until midsummer.

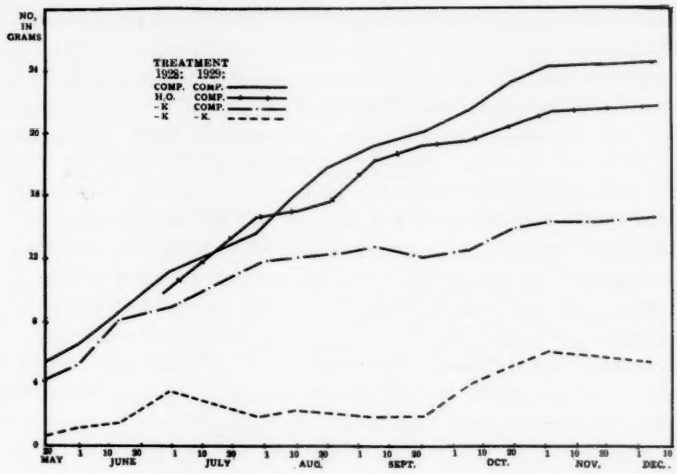

FIG. 6. Total nitrate absorption during season of 1929, by two-year-old French prune trees growing in various solutions.

Following the –S trees, the group in –K showed the next highest rate (and total) for nitrate absorption; it was nearly equal to that of the –S trees up to July 30, when the absorption of the –K trees fell off badly. In fact, loss of nitrate from the trees (by exosmosis into the culture solution) occurred before the close of the season. It should be noted that no loss of nitrate from trees in complete cultures occurred at any time of the season from March to December 1, and only slight losses during the winter months.

In spite of the fact that the group of trees in –K + Na made greater growth than those in –K, the nitrate absorption of the former was far less than that of the –K trees. The presence of sodium may actually be detrimental to nitrate absorption. During much of the season the –K + Na group showed a slower rate of nitrate intake than any other group of trees except the low-calcium group.

Groups of trees in –Mg and in –P differed only little from those in –K + Na in their nitrate absorption, all being very poor. This was true in spite of the fact that the –Mg trees made a terminal growth nearly equal to that made by the –S trees, although the fresh-weight increase and increase in trunk diameter (3 inches above the bud) were very much reduced in the case of –Mg trees. The –S trees were comparatively poor in trunk-diameter increase, making only one-half that made by complete-solution trees, although the total fresh-weight increase of the –S trees was equal to 85 per cent. of that made by the check trees. In short, the –S trees grew

unusually fine root systems, but top growth was not proportionally as good as root growth.

The absence of PO_4 did not diminish weight increase nearly as greatly as did magnesium starvation. The foliage of the –P trees during the first year (1929) was badly injured. The least gain in fresh weight occurred in the –Ca group of trees. The $-NO_3$ trees, in spite of very poor terminal growth, make proportionately very good weight increases, although both gains are small compared with the gains of complete-solution trees.

A group of French prune trees one year older than the preceding series was completely starved for two years, growing in distilled water; another group was grown in complete solution; a third group was grown in pure water the first year, and placed in complete solution the second year. Completely starved trees showed at the end of two years actual loss in trunk diameter, although considerable shoot growth had been made from reserves in the trees and the average fresh-weight increase per tree for the two years was about 160 gm., the initial fresh weight of the trees being on the average 164 gm.

Trees starved completely for one year, and then transplanted to complete solutions the second year, showed that terminal growth and total weight increase are made somewhat at the expense of diameter increase, which still lags notably behind for a year or so, until the tree has caught up with its salt absorption.

Also in the block of older French prune trees, to which reference has been made, was a group of trees starved for potassium over a two-year period. On April 12 of the third year of starvation, some of these trees were transferred to a +K solution, and developed excellent shoot growth and fine leaf color in about two weeks' time. Only a trace of the former chlorosis remained near the midrib portions of the leaf blade. The leaf color had markedly improved 5 days after the potassium was added to the solution. Later in the summer, the tip leaves of the young shoots of these same trees showed excessive reddening, then browning or burning and drying up. The shoot tip itself died back 6–8 inches.

Other French prune trees, also previously starved for potassium for two years, and then placed in complete-solution cultures on May 15, or on June 25 of the third year, showed no shoot growth response whatever to the added potassium. Leaf color did change in about ten to twelve days, and the live parts of badly scorched, chlorotic leaves turned green except for small areas bordering on the scorched margins of the leaf blades.

The roots of all of these trees responded quickly to added potassium, making abundant root growth. Yet it appears impossible to stimulate shoot growth in these trees by adding potassium after about May 1.

Conductivity measurements

The curves of specific resistance (figs. 7, 8) of the solutions of the various starvation groups are worthy of notice. Perhaps the most striking observation was that nitrate-starved trees, in spite of moderate, healthy root growth, were apparently losing certain nutrient salts to the external

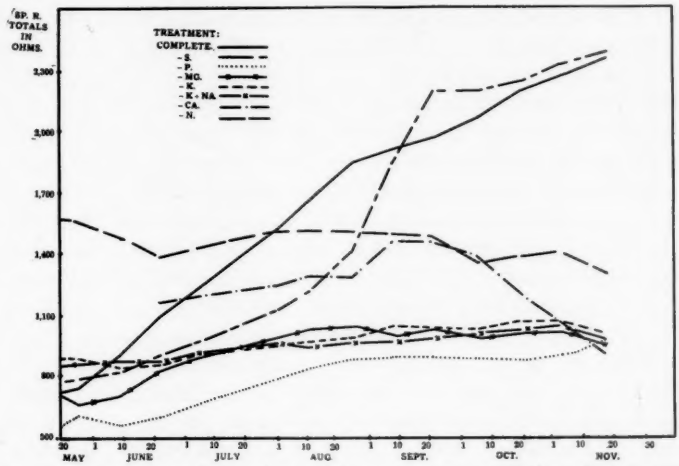

Fig. 7. Total change in specific resistance of culture solutions during season of 1929.

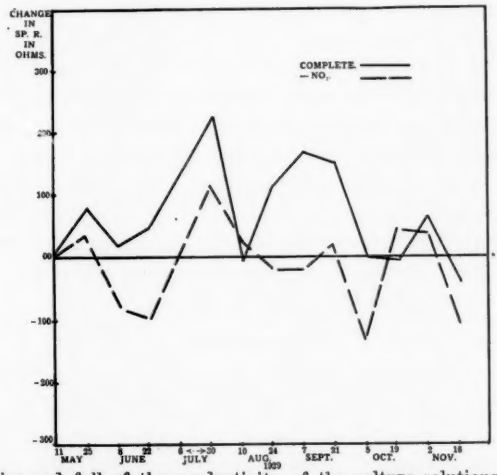

Fig. 8. Rise and fall of the conductivity of the culture solutions (change in conductivity in ohms, per two-week period). One-year-old French prune trees, season of 1929.

solution, throughout most of the season. The low-calcium trees, after calcium starvation became severe (these trees were fed calcium during the early summer to start root growth), appeared to lose an amount of nutrient salts nearly equal to the total previously absorbed in the same season. As mentioned, however, nitrate (perhaps already assimilated) was not the ion lost back to the solution in this period of exosmotic migration. Presumably sulphates, chlorides, phosphates, and bicarbonates were excreted or diffused back into the solution from dying rootlets. There seems to be no doubt that fruit tree roots starved for calcium made very poor nutrient absorbing systems. Apparently their usual properties of semipermeability are lost in such circumstances, and browning and rotting of the young root tips soon follow the change in permeability. On the other hand, it has been found that a solution of calcium hydrate alone, at pH 7.2–7.4, provides a very good medium for healthy root growth by fruit trees. As WOLFF showed long ago, young forest trees can be grown for long periods in calcium chloride solutions alone. HEILBRUNN's work on calcium and membrane "healing," permeability, protoplasmic vacuolation and streaming in amoeba, etc., is all pertinent to the problem, as is also FARR's (**11**) recent work. FARR has shown that for root-hair growth by such plants as the collard, *Brassica oleracea,* only calcium need be present in the external bathing solution. Calcium hydrate solution at pH 10 gave the fastest root-hair growth of any calcium salt used; and the maximum rate of root growth occurred at pH 8.0–8.5 in short-period experiments.

In the present work it has been found that it is entirely possible to transfer fruit trees in mid-season, from complete solution to simple $Ca(OH)_2$ solution of pH 7.2 or *vice versa,* without any apparent injury to the young white roots, or to older roots, and without any exosmosis of chlorides taking place. Outward diffusion of the latter usually occurs if the permeability of the tissues containing chlorides is appreciably altered from the normal. Such mid-season transfers as have been described make possible a new type of seasonal absorption work; the trees may be grown in complete solution for one, two, or three months, and then completely starved except for calcium during the rest of the year. Growth observations on such trees should show how dependent (or independent) shoot growth is on recently absorbed nutrient salts.

Summary

1. A review of the literature of water-culture studies with trees is presented, extending from the time of DUHAMEL DU MONCEAU in 1755 down to the present date.

2. The course of seasonal nitrate absorption, and of elemental starvation, was studied in young one and two-year-old French prune trees, as grown in water cultures in the greenhouse.

3. The effects of elemental starvation (for elements other than nitrogen) on seasonal and total-nitrate absorption are shown for trees of the French prune variety. Sulphate starvation appeared to have a far less depressing effect on nitrate absorption than did starvation for any other of the six major elements of the complete-culture solution. (Iron starvation was not studied in this series.) Potassium, magnesium, and phosphorus starvation all very seriously depressed nitrate absorption, even resulting in loss of nitrate from the roots, late in the season. Calcium starvation prevented root growth entirely. Low-calcium trees (fed a small amount of calcium) absorbed very little nitrate, and lost solutes from the roots when placed in a minus-calcium solution. The root tips of these trees invariably turned brown and died. Roots previously well stocked with calcium survived somewhat longer than the low-calcium roots when both were placed in minus-calcium solutions.

4. The total phosphate absorption for the season was far more badly depressed by magnesium starvation than by potassium or sulphur starvation. Calcium starvation apparently prevented absorption of any considerable quantity of any ion, including phosphate.

5. The primary peak in the seasonal nitrate absorption curve of the complete-solution French prune trees (both one and two-year-old groups) occurred near the end of June, in 1929; with a final, secondary peak falling in late October, followed by a rapid decline to winter dormancy. The complete-solution trees, in 1930, now two years old, again gave the maximum monthly total nitrate absorption in the period June 10–July 10, with brief periods of rapid nitrate absorption coming about May 1, August 20, and October 7. The curve for potassium absorption follows closely that for nitrate absorption, lagging slightly behind in the earlier part of the season.

6. There is a high correlation between temperature (probably light intensity also) and the rate of nitrate and potassium absorption by these trees.

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PHYSIOLOGICAL ANATOMY, TYPE, VARIETY, AND MATURITY OF CITRUS FRUITS AS AFFECTING QUALITY OF PREPARED JUICES

HAMILTON P. TRAUB, LEONARD W. GADDUM, A. F. CAMP, AND ARTHUR L. STAHL

(WITH ONE FIGURE)

Introduction

The anatomy of different citrus fruits varies with the type, the variety, the quality, and the degree of maturity of the material used, and these factors are of great importance in any consideration of the problems encountered in the preparation of citrus juices. It is of first importance to know how the anatomical structure and the physiological state of the material affect the development of undesirable tastes, changes in the color, and the settling of suspended particles in prepared juices on aging.

The preliminary results presented in this paper concern the localization in the complex tissues of the fruit of the causal agent or agents for changes observed in the prepared juice, and the effect of the citrus type, variety, and maturity factors on the physiological anatomy of the fruit used.

Plan of experiment

The causes of any undesirable changes in prepared citrus juices from the time of preparation until used may be classified under two heads: (a) those having their origin in the prepared juice itself as a result of the amounts and character of solutes and extractives released by the method of preparation; (b) those present in the external environment, such as gases (particularly oxygen), containers, temperature, and methods of storage. Such factors could operate independently or in combination.

A detailed description of the citrus fruit type is given by FAWCETT and LEE (**15**). In addition to the tissues mentioned by these authorities, the seeds should be indicated which are attached by means of placentae to the locular walls where these come in contact with the central axis of the fruit (fig. 1). It is also of interest to note that among the constituents of certain of the tissues various glucosides occur (**20, 24, 21, 46**), and it has been shown (**46**) that the glucoside content decreases with maturity. Pectic substances are also known to occur in citrus fruits.

Throughout its development the citrus fruit is continually changing in composition and, as a consequence, the extracted juice varies from sample to sample. In the whole fruit the juice is contained within the juice sacs, or vesicles, and does not come into contact with the various tissues that make up the remainder of the fruit structure. In processes of juice extraction, however, the various tissues are broken down and the juice mixed with

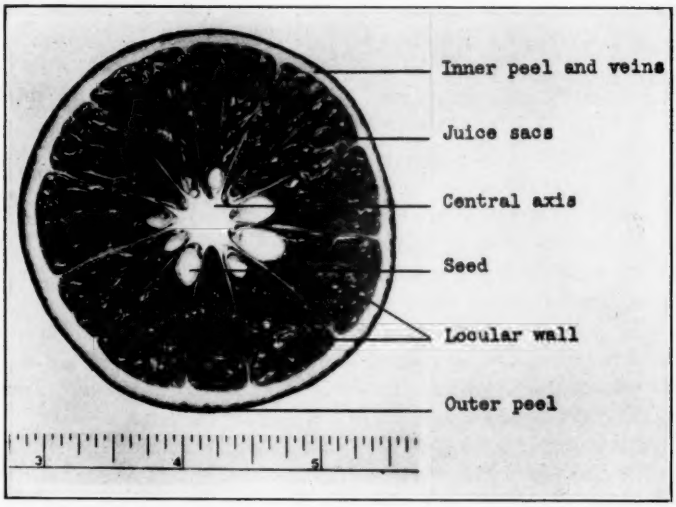

FIG. 1. Cross-sectional view of Parson Brown orange showing tissues.

them. Moreover, the extracted juice contains more or less of these macerated tissues in the form of solid particles and can take up from them any substances that they may contain.

Citrus juice extracted by the usual methods is a buffered solution with an effective acidity, according to reported experiments, ranging from pH 2.1 to pH 4.3. The more apparent solutes are total acids, 0.25 to 11 per cent.; total sugars, 1.5 to 16 per cent.; protein, 0.40 to 1.00 per cent.; and mineral salts, 0.20 to 0.40 per cent. (**38, 37, 28, 32, 33, 5, 10, 17**). In addition there are undoubtedly certain original solutes whose character has not been determined, as well as extracted solutes and suspended particles which originate mainly in the fruit tissues outside the juice sacs (**1, 3, 16**). These extracted solutes may include, among others, pectin, glucosides, aldehydes, and esters; and the suspended particles may consist of plastid pigments, citrus oil (in some methods of preparation), and macerated and torn tissue fragments of different sizes derived from the various tissues of the fruit. Extracted citrus juice apparently has a colloidal nature and as such shows marked individuality from sample to sample. Like most fluids of biological origin, the juice from citrus fruits is unstable or constantly changing in composition, but after extraction it has a tendency to become stable.

Materials and methods

PHYSIOLOGICAL ANATOMY

The fruit was separated into the component parts of its various tissues (**36**). The effect of these constituents when added to the relatively pure

juice was then determined. It was impracticable to separate single tissues, of course, and in carrying on the work groups of allied tissues were considered, such as locular walls, albedo (the inner peel), or outer peel, as shown in figure 1. In the discussion therefore each of these groups is referred to as a tissue. Eight such tissues were considered:

1. Outer peel (epidermal layer with some inner peel attached)
2. Inner peel and veins (albedo)
3. Locular walls
4. Central axis
5. Seed coat
6. Cotyledons and germ
7. Empty juice sacs
8. Juice expressed from filled juice sacs only

Infusions were made by grinding separately with sharp clean sand in a mortar equal portions by weight of the various tissues as listed. These infusions were then made up to volume and used as indicated later in this section. Certain of the constituents of the tissues were studied, particularly the oil, the oil-free sap from the outer peel, and the glucosides from the inner peel and veins.

Type, variety, and source of fruit

The fruits used were grown in various parts of Florida. Satsuma oranges were obtained from Glen St. Mary, Green Cove Springs, Pensacola, Marianna, and Gainesville; Parson Brown from Lake Alfred and Lady Lake; Hamlin from Grand Island; Washington Navel from Gainesville; and the Pineapple variety from Lake Alfred. Tangerines were obtained from Bartow, Hawthorne, and the Experiment Station at Gainesville; grapefruit from Lake Alfred and Gainesville; Key limes from Islamorada; Tahiti limes from Loughman, and a variant of the Tahiti lime from Homestead.

Maturity of fruit

Owing to the fact that the work herein reported was carried out during a comparatively short period early in the fruit season, it was impossible to obtain much information on maturity; however, every effort was made to select fruit in such a way as to cover this subject as thoroughly as possible. The samples of Satsuma fruits varied from under-ripe to over-ripe, and to a lesser extent the same was true of the Parson Brown. The Pineapple and Navel oranges and the tangerines used in most of the experiments were a little below full maturity. Since the variety factor is of extreme importance, as many varieties as possible were studied and compared.

Methods of preparing juices

Descriptions of the various methods of preparing citrus juices dealing chiefly with the methods of bottling and sterilization of the juice after sedi-

mentation and clarification (**13, 12, 7, 4**) or of carbonating the product (**18**) appear in the literature. The methods of preparing citrus juices to be preserved by the newer "frozen pack" (**2**) development as considered in this paper on the basis of the physiological anatomy of the material used may be characterized as follows:

A. Relatively small amounts of tissue introduced:
   (1) Little peel oil introduced
      Method no. 1
      Method no. 1a
   (2) More peel oil introduced
      Method no. 2
B. Relatively greater amounts of tissue introduced:
   (1) By grinding, but no oil
      Method no. 3
   (2) By reaming, and very small amounts of oil
      Method no. 4

METHOD NO. 1.—This was devised as a control method, in that it provided juice that was virtually free from all tissues except juice-sac constituents. To obtain the juice sacs, the outer rind containing the oil glands was first removed by peeling; the outer wall of the segments with all attached inner peel tissue was then pared off with a stainless steel knife; finally the segments were separated and the segment walls removed by inserting the knife blade at the base of the segment along the central axis and sliding it outward along the locular wall, thus separating it from the juice-sac tissues. The collected tissue was wrapped in clean gauze and pressed between porcelain plates. No metal except the blades of the knives used in paring and dissecting came in contact with the juice in this method.

METHOD NO. 1A.—A variation of the first method was used to some extent in studying the normal physiology of citrus fruits. The locules (segments) of the peeled halved fruits were pressed between porcelain plates and the pressed juice collected. Since the juice did not remain in contact with either the locular walls or the inner peel and veins for any great length of time when pressed only once, there was little possibility of the leaching out of constituents from the locular walls or any adhering tissue.

METHOD NO. 2.—The juice was pressed from the halved fruits by an inverted aluminum cup which held the fruit and pressed it against a metal form having the same shape as the cup. The clearance between the form and the cup was adjusted to a little more than the thickness of the peel. The shape of the pressing surfaces conformed to that of the fruit and all parts coming in contact with the juice were of aluminum alloy.

METHOD NO. 3.—The juice was pressed from the whole peeled fruit by means of a conical worm in a conical fluted housing having a strainer slot

along the bottom and a valve at the end through which the pulp passed. This press was made of iron heavily tinned, except on the edges of the worm and the flutings, and these were kept bright.

METHOD NO. 4.—The pulp of the fruit was reamed out on a high-speed revolving cone (**9**) of a stainless alloy and collected in a porcelain bowl.

AFTER-TREATMENT

In handling the juice after extraction and prior to storage the procedure was as follows: The juice was run directly from the extractor through a tinned wire strainer with 18 meshes to the inch and then into a glass receptacle from which the juice was transferred to a glass vacuum flask and vacuumized (**41**). A vacuum was kept on the flask, and as 200–300 cc. of juice were collected in the upper container it was transferred to the flask by opening the glass stopcock. In this way the juice was under vacuum within 2 or 3 minutes, at the longest, after it had been extracted. Juice extracted by method no. 4 was subject to excessive foaming during the vacuumizing process, which suggested the possibility that a great deal of air had been dissolved by the beating effect of this method. While the exact importance of the use of the vacuum is not established, it has the advantage in experimental work of giving samples of juice that contain approximately the same amounts of dissolved air, thus eliminating marked variations that might be due to unequal aeration. The vacuum in the flask was relieved with air admitted to the top of the flask (not bubbled through the juice).

The suitability of glass containers for frozen-pack work has been discussed by WOODROOF (**45**). After extraction and treatment the juice was placed in 8 or 10-oz. jelly glasses and capped under vacuum with lacquered caps. A vacuum of 27–29 inches was drawn on the container for an instant at the time of capping.[1] These containers were selected as the best type for experimental work because they eliminated the possible effects of paraffin, paper, air, and absorbed odors. The 8-oz. container was usually used and filled to 0.25 inch of the top.

The juice was frozen by immersing the glasses up to the lids in rapidly circulating brine, and then placing upon the lids a metal tank through which also brine was circulating. The brine temperature was maintained at $-20°$ to $-25°$ F., and the contents of the glasses were completely frozen in about 25 minutes. After freezing, the outsides of the glasses were rinsed in fresh water to remove the brine and they were stored at 0° F.

Some of the juice was put into similar containers, and without previous freezing was stored at 32° F. The samples of frozen juice were thawed by immersing the containers in running tap-water, and the caps were removed when thawing was completed. The fruit was washed prior to ex-

[1] Equipment for capping the containers lent by courtesy of the manufacturers.

tracting the juice and all containers and extractors were kept thoroughly clean but were not sterilized.

Physical and chemical determination

When juice was first extracted, detailed records were made of effective acidity (pH), total soluble acids, total sugars, and other physico-chemical characters. Such determinations were made again whenever samples were removed from storage for testing. The quinhydrone electrode was used in making the pH determinations (**35**). Total soluble acids were determined by titration with sodium hydroxide, using phenolphthalein as an indicator, corrections being made for temperature differences. The results are expressed as percentage by weight of anhydrous citric acid of the expressed juice.

Specific gravity was determined by means of an hydrometer reading to 0.001, corrections being made for temperature differences. The results are expressed at $\frac{60°}{60°}$ F.,[2] and have also been converted to the corresponding approximate values for degrees Brix for purposes of comparison with results appearing in the literature (**8, 11, 37, 38**).

Naringin was prepared from grapefruit according to the method of Zoller (**46**); hesperidin was prepared from the sweet orange according to the method of Wander (**42**). The words "glucoside" and "glucosidal" as used in this paper refer to the fractions extracted by these methods. In the case of the characteristic glucoside of Satsuma and of lime, the procedure of Wander was followed and the purified product obtained was assumed to be the glucoside.

Presentation of data

The principal changes observed in prepared citrus juices on aging as related to methods of preparation are those in taste, color, and movement of larger particles in the more or less translucent or transparent suspension medium. While working with Kawano Wase Satsuma oranges on October 19, 1931, a definite correlation was established between certain methods of preparation and the development of a bitter taste and a change in color (table I). In addition to these problems, changes in the direction and rate of movement of suspended particles challenged investigation (**39, 6**).

The effective acidity (pH) and the total soluble acids and solids of the mixture gave no indications as to the causes of the observed changes as shown by the data in table II. Although there were preceptible differences with regard to these characters, there seems to be no definite correlation between the observed changes in the juice and the differences mentioned.

[2] Indicates density at 60° F., referred to water at the same temperature.

TABLE I

CORRELATION BETWEEN METHOD OF PREPARATION AND DEVELOPMENT OF BITTER TASTE AND CHANGE IN COLOR; KAWANO WASE SATSUMA ORANGES, OCTOBER 19, 1931

METHOD OF PREPARATION*	CHANGE IN		REMARKS
	TASTE	COLOR	
No. 1a (check) †	Pleasantly tart; no bitter taste	Deep chrome (orange); no change	Retained good quality in refrigerator for several days
No. 2	Pleasantly tart with added flavor of citrus oil; no bitter taste	Color changed from deep chrome (orange) to sulphur (light yellow) within 1 hour	Did not develop bitter taste on standing at room temperature for 24 hours
No. 3	Developed bitter taste in 2 hours	Deep chrome (orange); no change	Taste became exceedingly bitter after 4 hours
No. 4	Developed bitter taste in 2 hours	Deep chrome (orange); no change	Taste became exceedingly bitter after 4 hours

* See text for descriptions of methods of preparation.

† On all later experiments, method no. 1 was used as the check treatment.

It was therefore necessary to make an attempt to localize the causes, either in the external environment or in the complex tissues of the fruit, or in a combination of these two factors. After localization of the causes the next logical step was to determine the factors involved in bringing about the changes.

The results are presented in order as follows: (a) changes in taste; (b) changes in color; and (c) changes in direction and rate of movement of suspended particles.

CHANGES IN TASTE

Undesirable tastes developed in prepared citrus juices on aging are described as *stale, bitter, limy, musty,* and *"turpentine"* in the literature as reported up to 1925 (**28, 13**). These terms may not be strictly applicable to the product put up under the new frozen-pack method. In the case of juices put up in glass containers by the new method, one of the chief difficulties encountered up to the present is the development of a bitter taste (**9**). As shown in tables I and IX, this bitter taste develops relatively soon after the extraction of the juice, if the method of preparation is a predisposing factor. However, such other factors as the temperature of storage and the maturity of the fruit must also be considered in this connection. While other undesirable flavors may develop in the frozen or cold-stored juice, this preliminary report deals only with the bitter taste.

TASTING TECHNIQUE.—As a rule the results presented are based upon the tasting of the juices by two or more individuals. An attempt was

TABLE II

CHARACTERISTICS OF CITRUS JUICES AND THE EFFECT OF AFTER-TREATMENT

TYPE OF CITRUS FRUIT	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	STORAGE PERIOD	REACTION	TOTAL ACIDS	SPECIFIC GRAVITY	DEGREES BRIX
				days	*pH*	*per cent.*		
Satsuma (Owari)*	11/19/31	1	Original juice	0	3.50	1.020	1.037	9.3
" "	"	1	Frozen	6	3.51	0.996	1.036	9.0
" "	"	2	Original juice	0	3.52	1.000	1.037	9.3
" † "	"	2	Stored at 32° F.	6	3.63	0.980	1.038	9.6
" "	"	2	Frozen	6	3.46	0.980	1.037	9.3
" "	"	3	Original juice	0	3.58	1.020	1.036	9.0
" "	"	3	Stored at 32° F.	6	3.59	0.854	1.037	9.3
" "	"	3	Frozen	6	3.59	0.860	1.037	9.3
" "	"	4	Original juice	0	3.55	0.972	1.038	9.6
" "	"	4	Stored at 32° F.	6	3.53	0.942	1.038	9.6
" "	"	4	Frozen	6	3.54	0.935	1.037	9.3
Orange (Pineapple)*	11/23/31	1	Original juice	0	3.46	1.260	1.047	11.7
" "	"	2	Original juice	0	3.47	1.250	1.047	11.7
" "	"	2	Stored at 32° F.	2	3.41	1.490	1.048	12.0
" "	"	2	Frozen	2	3.45	1.380	1.047	11.7
" "	"	3	Original juice	0	3.47	1.390	1.047	11.7
" "	"	3	Stored at 32° F.	2	3.48	1.360	1.048	12.0
" "	"	3	Frozen	2	3.38	1.330	1.048	12.0
" "	"	4	Original juice	0	3.45	1.390	1.047	11.7
" "	"	4	Stored at 32° F.	2	3.40	1.430	1.048	12.0
" "	"	4	Frozen	2	3.41	1.360	1.045	11.2
Tangerine (Dancy)	11/23/31	2	Original juice	0	2.95	2.150	1.048	12.0
" "	"	2	Stored at 32° F.	4	2.95		1.047	11.7
" "	"	2	Frozen	4	3.02	2.060	1.045	11.2
" "	"	3	Original juice	0	2.96	2.060	1.048	12.0
" "	"	3	Stored at 32° F.	4	3.07	2.010	1.047	11.7
" "	"	3	Frozen	4	3.09	2.010	1.045	11.2
" "	"	4	Original juice	0	3.03	2.120	1.048	12.0
" "	"	4	Stored at 32° F.	4	3.01	2.000	1.048	12.0
" "	"	4	Frozen	4	3.04	2.030	1.045	11.2
Lime (Key)*	7/23/31	2	Frozen	120	2.26	7.080	1.037	9.3
" "	"	3	Frozen	120	2.31	6.650	1.037	9.3
" "	"	4	Frozen	120	2.32	6.870	1.038	9.6

* Similar data were compiled for Kawano Wase Satsuma; Valencia, Parson Brown, Washington Navel, and Hamlin Sweet Orange; and Tahiti lime.

† Two samples of shorter storage period gave same results.

made to use constant quantities of materials for tasting purposes in any particular experiment. The degrees of taste differences are indicated on a relative basis only. When any particular taste was present in a pronounced degree, it is indicated by a (+) sign. Decreasing degrees of any particular taste are indicated by (+−), (+−−), etc.; increasing degrees are indicated by (++), (+++), etc.

BITTER TASTE.—A clue to the localized tissues responsible for the bitter taste which develops in citrus juice prepared by certain methods was definitely shown in preliminary experiments (**39, 6**). The difference in taste was found to be due to the method of preparation, and this indicated that the cause of the trouble could possibly be traced to certain of the complex tissues of the fruit. While still in the whole fruit, these various tissues are effectively separated from one another. As has been indicated, however, the juice extraction process tears down these barriers more or less completely and breaks up the tissues, depending on the method used, and the resulting juice may be a mixture of material from most of the tissues. Once the juice is prepared in a manner predisposed to the development of the bitter taste, other factors may enter which may delay or modify the degree of its development under any particular conditions of after-treatment.

Although it was suspected that the causal agent might be of glucosidal nature, the plan of procedure included a comprehensive study of other possible causes. The following lines of investigation were followed in an attempt to determine the source of the undesirable tastes: (a) localization of the cause in the tissues; (b) determination of the nature of the mechanism involved (whether enzymatic, non-enzymatic, solution, etc.); (c) determination of effect of variety, maturity, and quality of fruit; and (d) determination of effect of after-treatment of the juice (freezing, storage, etc.).

LOCALIZATION OF CAUSAL AGENT OR AGENTS.—It was necessary to consider as many of the tissues of the fruit as possible so that no important source of the trouble would escape investigation. The tissues which were obviously of no importance were quickly eliminated, but a more detailed study of the remaining tissues was made with the object of localizing the cause or causes in a definite region or regions in the fruit.

To eliminate from the study the tissues which did not seem to contain the causal agent of the bitter taste, infusions were made by adding macerated tissues to the juice and testing for the effect on bitter taste development. The check treatments consisted of similar dilutions using tissue infusions made with water. Since the degree of dilution necessary to secure any marked results was unknown, three degrees of dilution were included in the first experiment, 2, 4, and 8 parts of macerated tissue to 100 parts of juice pressed from juice sacs only.

Sweet Orange; and Tahiti lime.
† Two samples of shorter storage period gave same results.

TABLE III

LOCALIZATION IN TISSUES OF SATSUMA ORANGE FRUIT (KAWANO WASE) OF THE CAUSE OF THE BITTER TASTE, OCTOBER 28, 1931 (DILUTIONS MADE WITH JUICE PRESSED FROM JUICE SACS ONLY)

INFUSION		PARTS INFUSION PER 100 PARTS LIQUID	BITTER TASTE DEVELOPMENTS*	
TISSUE	EXTRACTIVE USED		IMMEDIATE	LATER
Outer peel	Water	2	−	−
"	"	4	−	−
"	"	8	+−	+−
" "	Juice	2	−	−
"	"	4	+−	+−
"	"	8	+−	+
Inner peel and veins	Water	2	+−	+−
"	"	4	+−	+−
"	"	8	+	+
" " " "	Juice	2	+−	+−
"	"	4	+−	+−
"	"	8	+	+
Locular walls	Water	2	+−−	+−
"	"	4	+−	+
"	"	8	+	++
" "	Juice	2	+−	+−
"	"	4	+−	+−
"	"	8	+	+
Central axis	Water	2	−	−
"	"	4	−	−
"	"	8	−	−
" "	Juice	2	−	−
"	"	4	−	−
"	"	8	−	−
Seed coat	Water	2	−	−
"	"	4	−	−
"	"	8	−	−
" "	Juice	2	−	−
"	"	4	−	−
"	"	8	−	−
Cotyledons and germs	Water	2	−	−
"	"	4	−	−
"	"	8	−	−
" " "	Juice	2	−	−
"	"	4	−	−
"	"	8	−	−

* Relative taste differences are indicated as follows: (−) indicates absence of the bitter taste; (+−), (+), (++), etc., indicate relatively greater degrees of the bitter taste.

The data in table III show that the tissues most probably responsible for the development of the bitter taste are the inner peel and veins, the outer peel, and the locular walls (**3**). The respective degrees of bitterness developed under the experimental conditions (dilution 8 parts of macerated tissue to 100 parts of juice) were for the freshly prepared infusion of outer peel (+−), and for the same infusion after standing (+); for the inner peel and locular walls (+) when freshly prepared infusion

was tested, and (+) for the same infusion after standing. The tastes given by the seed coat and cotyledons plus germs were of a nature different from the one being investigated; the central axis and juice-sac tissues were practically tasteless excepting in certain varieties of such citrus types as the grapefruit (tables VI, VII, and VIII). In this fruit the vascular tissues connecting with the juice sacs are much coarser than in some other citrus types, and the juice expressed from the separated locules thus carries more of the material commonly associated with the inner peel and locular wall. The juice sacs themselves are relatively coarse in structure and may contain the bitter principle. Similar results for the grapefruit have been reported by FELLERS (**16**). After the elimination of other tissues, the work was concentrated on the tissues of the inner peel, locular walls, and outer peel.

In further experiments, infusions made with outer peel from which the adhering portions of the inner peel had been more carefully removed failed to give a detectable bitter taste, although this taste may have been present but masked by the oil taste. As a result of these experiments, the outer peel was eliminated from the study as not being important in producing the bitter taste. Up to this point all the fruit used was below full maturity. In later experiments with riper fruit it appeared that the locular walls were probably less important as a source of the bitter taste than were the inner peel and the vein tissues.

The next step was to determine the factors involved in bringing about undesirable taste developments in prepared citrus juices from the standpoint of the presence or absence of inner peel and veins or locular-wall tissue.

The tests for the enzymatic factors proved negative. The work of REED (**34**) and MCDERMOTT (**41**) on the inactivation of oxidases and peroxidases in citrus juices was verified as shown in table IV. The experiment included a study of: (a) the distribution of oxidase and peroxidase in the locular walls, inner peel and veins, and outer peel (**29**); (b) the effectiveness of water and juice in extracting possible enzymes; (c) the effect of Berkefeld filtering and centrifuging on tissue extracts made with water and juice; and (d) the effect on oxidase and peroxidase activity of mixing water extract of inner peel plus veins (giving positive reaction for oxidase and peroxidase) with the original juice, Berkefeld-filtered juice, and centrifuged juice.

In these experiments, all water extracts, untreated, Berkefeld-filtered, and centrifuged, gave positive evidence of oxidase and peroxidase activity; however, the last two gave color reactions to a less degree. Juice extracts as a general rule gave either negative or contradictory results; untreated juice and centrifuged juice extracts gave negative tests for these enzymes,

TABLE IV

REGIONAL DISTRIBUTION OF OXIDASE AND PEROXIDASE IN THE TISSUES OF THE SATSUMA ORANGE FRUIT (KAWANO WASE); NOVEMBER, 2, 1931

INFUSION*			OXIDASE (GUAIACUM TEST)		PEROXIDASE (GUAIACUM TEST)		REMARKS
TISSUE EXTRACTED	EXTRACTANT USED	AFTER-TREATMENT	NOT BOILED	CHECK (BOILED)	NOT BOILED	CHECK (BOILED)	
Locular walls	Water	Untreated	Blue	Negative	Intense blue	Negative	
"	"	Berkefeld-filtered	Light blue	"	Medium blue	"	
"	"	Centrifuged	Faint blue	"	Medium blue	"	
"	Juice	Untreated	Negative	"	Negative	"	
"	"	Berkefeld-filtered	Faint blue	"	Light blue	"	Same filter used as in water sample
"	"	Centrifuged	Negative	"	Negative	"	
Inner peel and veins (a)	Water	Untreated	Blue	"	Intense blue	"	
"	"	Berkefeld-filtered	Faint blue	"	Very light blue	"	
"	"	Centrifuged	Faint blue	"	Medium blue	"	
"	Juice	Untreated	Negative	"	Negative	"	
"	"	Berkefeld-filtered	"	"	"	"	Not kept in cold storage
"	"	Centrifuged	"	"	"	"	Not kept in cold storage
Outer peel	Water	Untreated	Faint blue	"	Intense blue	"	
"	"	Centrifuged	Light blue	"	Intense blue	"	
"	Juice	Untreated	Negative	"	Medium blue	"	
"	"	Centrifuged	"	"	Medium blue	"	
Other mixtures tested							
Original juice (b)		Berkefeld-filtered	"	"	Negative	"	
Original juice (c)		Centrifuged	"	"	"	"	
" (d)		Untreated	"	"	"	"	
Juice poured off parings of inner peel and veins			"	"	"	"	
Juice poured off locular walls			"	"	"	"	
Mixture of (a) and (b)†			"	"	"	"	
" (a) and (c)†			"	"	"	"	
" (a) and (d)†			"	"	"	"	

* Infusions made in the proportions of 1 gm. of fresh tissue to 1 cc. extractant.
† Dilution: 1 part infusion to 8 parts of juice.

except in the case of outer peel extract; and Berkefeld-filtered juice extract gave contradictory results. Water extracts, whether Berkefeld-filtered, centrifuged, or untreated, when mixed with original Satsuma juice gave no indication of the presence of enzymes, indicating that the degree of acidity (pH 3.5 in this case) inactivates the enzymes present in the tissue extracts.

The presence of glucosidases was investigated from the viewpoint of possible change in optical activity of clarified juice from juice sacs only. When tissue infusions possibly containing glucoside-splitting enzymes were added to this clarified juice (**19**), any changes in optical activity observed were well within the probable error of the experiment.

The possible relation between other enzymes and the development of bitter taste was tested by adding boiled tissue to unboiled juice, *vice versa*, and also by boiling both before mixing. The tests gave negative results as shown in table V. There was no decrease but rather an increase of the bitter taste due to boiling.

TABLE V

TESTS TO DETERMINE THE NATURE (ENZYMATIC OR NON-ENZYMATIC) OF THE BITTER TASTE DEVELOPMENT IN CITRUS JUICES

INFUSION		BITTER TASTE* DEVELOPED	
EXTRACTANT USED	INNER PEEL AND VEINS ADDED	SATSUMA ORANGE (KAWANO WASE) NOV. 9, 1931	SWEET ORANGE (PARSON BROWN) NOV. 10, 1931
	gm.		
Juice†	None	−	−
"	0.01	−	+−
"	0.10	−	+−
"	1.00	+	+
"	5.00	++	++
"	1.00 boiled‡	+	+
Boiled juice† ‡	None	− (+ boiled taste)	− (++ boiled taste)
" "	1.00	++ (+ boiled taste)	+− (++ boiled taste)
" "	1.00 boiled‡	+++ (+ boiled taste)	++ (++ boiled taste)
Water	None	−	−
"	0.01	+−	+−−
"	0.10	+	+
"	1.00	++	+
"	5.00	+++	++
"	1.00 boiled‡	++	+++

* Relative taste differences are indicated as follows: (−) indicates absence of the bitter taste; (+−), (+), (++), etc., indicate increasing degrees of bitter taste. Data taken within 15 minutes after mixing and re-checked after 24 hours.

† Juice pressed from juice sacs only.

‡ Heated to boiling point.

* Infusions made in the proportions of 1 gm. of fresh tissue to 1 cc. extractant.
† Dilution: 1 part infusion to 8 parts of juice.

The evidence apparently points to the possibility of a non-enzymatic cause for bitterness. Experiments to test this hypothesis were conducted along four lines: (a) testing the possible identity of the causal agent for bitter taste with glucosides; (b) determining the effect on taste of varying amounts of glucoside; (c) determining the effect of the extraction pressure and the temperature factors on the degree of bitter taste developed in prepared citrus juices; and (d) determining the solubility of the substance causing the bitter taste.

Aliquots of the tissue extracts were centrifuged and Berkefeld-filtered; similarly, aliquots of the juice used for dilution in the case of locular walls and inner-peel and vein infusions were centrifuged and Berkefeld-filtered. The centrifuging and Berkefeld-filtering processes did not seem to remove the causes of bitterness, since samples consisting of Berkefeld-filtered infusions diluted with similarly treated juice expressed from juice sacs only still gave mixtures which developed a bitter taste. As a result of these experiments it appeared that the cause of the bitter taste was a soluble substance.

When the experiments were undertaken, it was suspected that the causal agent for the bitter taste was of glucosidal origin; however, it was necessary to eliminate other possibilities in order to obtain reasonable assurance that the original hypothesis might prove tenable.

The data presented in tables V and VI indicate that the degree of bitterness developed when inner-peel and vein tissue or naringin is added to Satsuma juice pressed from juice sacs only is in a marked degree proportional, within the limits of the experiment, to the amount of inner-peel and vein tissue or glucoside added to juice expressed from juice sacs only. In the case of grapefruit, the bitter taste is present even in juice expressed from juice sacs only, but the degree of the characteristic bitterness may be increased by adding naringin, as shown in table VI.

A more comprehensive experiment which verifies these conclusions was carried out with juice of Satsuma orange, sweet orange, grapefruit, lime, and tangerine, as presented in table VII. In the experiment the juice from juice sacs only was treated in two ways: (a) by addition of the glucoside extracted from the inner peel and veins; (b) by addition of the inner peel and veins from which the glucosides had been extracted. The data show that the extracted tissues gave negative results except in the case of grapefruit juice, which result is in harmony with previous experiments. The addition of the extracted glucoside gave positive results, the degree of bitterness increasing with the amount of glucoside added.

The data presented in tables V, VI, and VII indicate that the major cause of the bitter taste developed in prepared citrus juices on aging apparently is of glucosidal origin. Since the chemical structure of the

TABLE VI

POSSIBLE IDENTITY OF CAUSAL AGENT FOR BITTER TASTE WITH GLUCOSIDE; JUICE PRESSED FROM JUICE SACS ONLY

SOLUTION		BITTER TASTE* DEVELOPED		
SOLVENT USED	DEGREE SATURATION OF NARINGIN SOLUTION†	SATSUMA ORANGE (KAWANO WASE) 11–10–31‡	GRAPEFRUIT (SILVER CLUSTER) §	
			11–18–31	11–20–31
Juice	None	−	+	++
"	0.0001	−	+	+++
"	0.001	−	++	+++
"	0.01	+−	+++	+++
"	0.1	+	++++	++++
"	Saturated	+++	++++	+++++
Boiled juice	0.0001	− (+ boiled taste)	+ (+ boiled taste)	+ (+ boiled taste)
" "	0.001	− (+ " ")		
" "	0.01	+ (+ " ")		
" "	0.1	++ (+ " ")	++++ (+ boiled taste)	++++ (++ boiled taste)
" "	Saturated	+++ (+ " ")		
Water	None	−	−	−
"	0.0001	+−	+−	+−
"	0.001	+	+−	+
"	0.01	++	+	++
"	0.1	+++	++	+++
"	Saturated	++++	+++	++++

* Relative taste differences are indicated as follows: (−) indicates absence of the bitter taste; (+−), (+), (++), etc., indicates relatively greater degrees of the bitter taste.

† Naringin prepared according to method of ZOLLER (45).

‡ Experiment set up November 9, 1931.

§ First one-third pressings used in this experiment.

TABLE VII

EFFECT OF CHARACTERISTIC GLUCOSIDE AND GLUCOSIDE-FREE TISSUE ON THE DEVELOPMENT OF THE BITTER TASTE IN PREPARED CITRUS JUICES.*
JUICE PRESSED FROM JUICE SACS ONLY

VARIETY	SOLVENT	TASTE OF UNTREATED SOLVENT	TASTES DEVELOPED WHEN VARYING AMOUNTS OF GLUCOSDE WERE ADDED			TASTES DEVELOPED WHEN VARYING AMOUNTS OF GLUCOSIDE-FREE TISSUE WERE ADDED			
			1/100 SATURATED	1/10 SATURATED	SATURATED	WEIGHT	TASTE	WEIGHT	TASTE
						gm.		*gm.*	
Satsuma (Owari) Dec. 4, 1931	Juice	–	+	+	+	0.01	–	0.25	–
	Water	–	+– –	+	++	0.01	–	0.25	–
Orange (Parson Brown) Nov. 12, 1931	Juice	–	–	+–	+	0.10	–	1.00	–†
	Water	–	–	+–	+	0.10	–	1.00	–
Orange (Hamlin) Dec. 4, 1931	Juice	–	+– –	+– –	+– –	0.25	–	1.00	–
	Water	–	+– –	+–	+	0.25	–	1.00	–
Grapefruit (Silver Cluster) Dec. 4, 1931	Juice	+	+	++	++++	0.25	+	1.00	+
	Water	–	+	++	++++	0.25	–	1.00	–
Lime (Key) Dec. 4, 1931	Juice	–	+	++	++++	0.01	–	0.25	–
	Water	–	+–	+++	++++	0.01	–	0.25	–
Tangerine‡ (Dancy) Dec. 4, 1931	Juice	–	+				–		–
	Water	–	+	++	++++	0.25	–	1.00	–

* Relative taste differences are indicated as follows: (–) indicates absence of the bitter taste; (+–), (+), (++), etc., indicate relatively greater degrees of the bitter taste.

† Tissue before extracting (1 gm.) gave +.

‡ Naringin was used; characteristic tangerine taste present in all juices.

glucosides contained in various types of citrus is not the same, it would be expected that the bitter tastes associated with them would not be identical. This is actually the case; and so far as the work was carried, each glucoside or closely related group can be recognized by its characteristic taste qualities.

The data presented in tables V and VI show also that the temperature factor may be of importance with reference to the rate of bitter taste development and the final value attained. The figures in table V show that the degree of bitter taste developed is markedly greater in cases where either the juice, the tissue, or both are heated before or after mixing. The figures presented in table VI, although not so conclusive as those presented in table V, show that apparently more naringin goes into solution as the temperature is raised, as indicated by increased bitterness of taste after boiling. This is in harmony with the work of ZOLLER (**46**), who has shown that naringin is more water-soluble at higher temperatures.

The data presented in table VIII indicate that the degree of bitterness developed when juice is expressed from inner-peel and vein tissue with attached juice vesicles by means of porcelain plates is in a marked degree correlated with the pressure exerted. The first 100 cc. develop the least bitter taste and usually the degree of bitterness rises markedly in the case of the third 100 cc. (**14**). It should be pointed out that when juices are expressed from juice sacs only, except in the case of the grapefruit, a bitter taste does not normally develop, even in 8 or 9 days in cool storage. This would indicate that the data in table VIII possibly should be interpreted on the basis that the bitter taste is due to dissolved glucosidal material pressed primarily from the cells of the inner peel and veins and locular wall tissues.

TYPE, VARIETY, QUALITY, AND MATURITY FACTORS.—The data presented in tables I to VIII, inclusive, are in harmony with the commonly accepted ideas regarding the wide differences in kind of juice secured from various types of citrus fruits, such as Satsuma orange, sweet orange, tangerine, grapefruit, and lime. The chief variations, such as effective acidity (pH), total soluble acids and solids, as well as the more subtle taste qualities associated with each type, are due to the kind of citrus fruit used. Within a citrus type the effect of variety on the quality of the juice is of great importance.

In the case of the Satsuma orange, two varieties have been studied. With juice prepared on October 19 from Kawano Wase Satsuma oranges (table I) a marked bitter taste developed within two hours. With juice of Owari satsumas the results were somewhat different. Juices were prepared on November 19 from fruits of average maturity from Marianna (table IX), and fruits of advanced maturity from the station orchard at Gainesville on

TABLE VIII

EFFECT OF VARIOUS DEGREES OF PRESSURE AND STAGE OF MATURITY ON DEVELOPMENT OF BITTER TASTE IN PREPARED CITRUS JUICES

TYPE OF CITRUS FRUIT	DATE	STATE OF MATURITY	NATURE OF TISSUE	PRESSINGS†	TASTE DEVELOPMENTS*				
					INITIAL	AFTER 4 HRS.	AFTER 8 HRS.	AFTER 12 HRS.	AFTER 20 HRS.‡
Satsuma (Owari)	Dec. 3, 1931	Relatively mature	Inner peel and and locular walls	1st	−	−	+−−	+−−	+−
				2nd	+−−	+−−	+−−	+−−	+
				3rd	+−−	+−−	+−	+−	+
Orange (Hamlin)	"	" "	Locular walls	1st	−	−	−	−	−
				2nd	−	−	−	−	−
				3rd	−	−	−	−	−
" "	"	" "	Inner peel and veins	1st	−	−	−		+−−
				2nd	−	−	−		+−−
				3rd	−	−	−		+−−
" "	"	" "	Locular walls and inner peel and veins	1st	−	−	−		+−−
				2nd	−	−	−		+−−
				3rd	−	−	−		+−−
Tangerine (Dancy)	Dec. 3, 1931	Relatively immature	Locular walls	1st	+	++	++		+++
				2nd	+	++	++		+++
				3rd	++	+++	+++		++++
" "	"	" "	Inner peel and veins	1st	+−	+	++		++
				2nd	+	+++	+++		+++
				3rd	+	+++	+++		+++
Grapefruit (Walters)	Nov. 18, 1931	Relatively immature (from Station grounds)	Juice sacs only	1st	+				++
				2nd	++++				++++
				3rd	++++++				++++++

* Relative taste differences are indicated as follows: (−) indicates absence of a bitter taste; (+−), (+), (++), etc., indicate relatively greater degrees of the bitter taste.

†1st indicates first 100 cc. of one pressing; 2nd indicates the second 100 cc. of the same pressing; 3rd indicates juice sample obtained by repressing. Pressings made between procelain plates.

‡ Juice stored at 40° to 50° F.

December 3 (table VIII). In the sample of average maturity prepared by methods 3 and 4 a bitter taste was developed to the degree of (+) at the end of two and three hours, respectively (table IX); the sample of advanced maturity gave a bitter taste only to the degree (+ – –) even when the juice was pressed from inner-peel and vein parings as indicated in table VIII.

As shown in table IX, the Washington Navel is more likely to develop the bitter taste than Parson Brown, Pineapple, or Hamlin. In grapefruit the glucoside taste is expected and there is even a popular prejudice against such a variety as Triumph which does not have the characteristic glucoside bitterness of this type of citrus. The study of the effect of quality of fruit within a variety in this particular has not been undertaken experimentally.

In most cases the bitter taste developed in sweet orange juice was found to be relatively little and was found to decline with advancing maturity. In table IX it will be noted that on November 13 and 14 two samples of juice were prepared from Parson Brown oranges, the first with fruit from Lady Lake, Florida, and the second with much less mature fruit from Lake Alfred. The first sample developed no noticeable bitter taste but the second sample developed a degree of bitterness with methods 3 and 4 of (+ –) after two hours of standing, indicating the effect of maturity in decreasing bitterness. Pineapple and Hamlin orange juice prepared by methods 3 and 4 gave results very similar to these. Juice prepared from the Washington Navel variety, however, on November 14 and 19 developed a degree of bitterness with methods 3 and 4 of (+ +) and (+), respectively, after two hours' standing on the first date and a degree of bitterness amounting to (+) with both methods after standing for five hours for the second date (table IX).

The tangerine juices prepared on November 23 gave results closely paralleling those for satsumas, developing a very bitter taste for methods nos. 3 and 4 after standing for several hours (tables I, VIII, and IX). Tahiti and lime juices prepared on November 18 by methods 3 and 4 developed a disagreeably bitter taste (table IX).

The results discussed are of course a mere beginning toward the complete listing of citrus varieties suitable for juice production on the basis of glucoside content and other constituents and their relative proportions remaining after various stages of maturity. The subject is of major importance and will require comprehensive experiments.

The preliminary results are in harmony with those often quoted in the literature to the effect that a bitter taste does not always develop or that properly matured fruit does not give a poor quality juice (**28, 9, 12**). The experiments here reported support these empirical statements and will make it possible after the subject is thoroughly investigated to select citrus fruits intelligently so as to minimize the bitter taste in the product. In determining the exact range in the maturity of citrus fruits when materials should

Pressings made between procelain plates.
‡ Juice stored at 40° to 50° F.

TABLE IX

BITTER TASTE DEVELOPMENTS IN PREPARED CITRUS JUICES IN RELATION TO METHODS OF PREPARATION, FREEZING, AND STORAGE*

TYPE OF CITRUS FRUIT	DATE PREPARED (1931)	METHOD OF PREPARATION	AFTER-TREATMENT	STORAGE PERIOD	BITTER TASTE DEVELOPMENTS AT DIFFERENT SAMPLINGS. FIGS. IN ( ) REFER TO HOURS AFTER EXPERIMENT WAS SET UP					
					1ST	2ND	3RD	4TH	5TH	6TH
				days						
Satsuma (Owari)	Nov. 19	1	Original juice	0	−	(½)−		(3)−		(16)−
"	"	1	Frozen	6	−	(½)−		(3)−	(5)−	
"	"	2	Original juice	0	−		(2)−			
"	"	2	Stored at 32° F.	6	−	(½)−	(1¾)−	(3)−	(4)−	
"	"	2	Frozen	"	−	(½)−		(3)−	(4)+−−−	
"	"	3	Original juice	0	−	(¾)+−	(2)+			
"	"	3	Stored at 32° F.	6	+−	(¼)+−	(1¼)+	(3)++	(5)+++	
"	"	3	Frozen	"	+−−	(¼)+−		(3)+−	(5)+−	
"	"	4	Original juice	0	−			(3)+	(5)+	
"	"	4	Stored at 32° F.	6	+−	(¼)+	(1¼)+	(3)++	(5)++	
"	"	4	Frozen	6	−		(1¼)+−	(3)+−	(5)+−	
Satsuma (Wase)	Nov. 13	2	Original juice	0	−	(2)−				
"	"	2	Stored at 32° F.	2	−	(3)−				
"	"	3	Original juice	0	+	(2)++				
"	"	3	Stored at 32° F.	2	++	(3)+++				
"	"	4	Original juice	0	++	(2)++				
"	"	4	Stored at 32° F.	2	+−	(3)+−				
Orange (Parson Brown) Lady Lake, Fla.	Nov. 13	2	Original juice	0	−	(2)−				
"	"	2	Stored at 32° F.	2	−		(3)−			
"	"	3	Original juice	0	−	(2)−				
"	"	3	Stored at 32° F.	2	−		(3)−			
"	"	4	Original juice	0	−	(2)−				
"	"	4	Stored at 32° F.	2	−		(3)−			
Orange (Parson Brown) Lake Alfred, Fla.	Nov. 14	2	Original juice	0	−	(2)−				
"	"	2	Stored at 32° F.	2	−		(3)−			
"	"	3	Original juice	0	+−−	(2)+−				
"	"	3	Stored at 32° F.	2	+−		(3)+			
"	"	4	Original juice	0	+−−	(2)+−				
"	"	4	Stored at 32° F.	2	−		(3)+−			

TABLE IX—(*Continued*)

Type of citrus fruit	Date prepared (1931)	Method of preparation	After-treatment	Storage period	Bitter taste developments at different samplings. Figs. in ( ) refer to hours after experiment was set up					
					1st	2nd	3rd	4th	5th	6th
Orange (Washington Navel)	Nov. 19	2	Original juice	0	−		(1)−	(5)−	(6)−	
"	"	2	Stored at 32° F.	4	−			(5)−		
"	"	2	Frozen	6	−		(1)+− −	(5)+−		
"	"	3	Original juice	0	−	(½)+−		(5)+	(6)++	
"	"	3	Stored at 32° F.	4	++		(2)++			
"	"	3	Frozen	6	+	(½)+	(3)++	(5)+++		
"	"	4	Original juice	0	+−	(½)+−		(5)+	(6)++	
"	"	4	Stored at 32° F.	4	++		(2)+++			
"	"	4	Frozen	6	+	(½)+	(2)++	(5)+++		
Tangerine (Dancy)	Nov. 23	1	Original juice	0	−	(4)−				
"	"	2	"	0	−	(½)−	(5)−	(7)−		
"	"	2	Stored at 32° F.	4	−	(¾)−	(1)−	(3)−		
"	"	2	Frozen	4	−	(¾)−	(1)+− −	(3)+−		
"	"	3	Original juice	0	+− −	(¼)+	(6)++++			
"	"	3	Stored at 32° F.	4	+	(¾)+	(1)+	(3)+		
"	"	3	Frozen	4	+	(¾)++	(1)++	(3)+++		
"	"	4	Original juice	0	+	(¼)++	(1)++	(7)+++		
"	"	4	Stored at 32° F.	4	+	(¾)++	(1)+	(3)+		
"	"	4	Frozen	4	+−	(¾)++	(1)+	(3)++		
Lime (Tahiti)†	Nov. 18	2	Original juice	0	−					
"	"	3	"	0	+					
"	"	4	"	0	+−					
Lime (Key)†	"	2	"	0	−					
"	"	3	"	0	+					
"	"	4	"	0	+−					

* Relative taste differences are indicated as follows: (−) indicates absence of a bitter taste; (+−), (+), (++), etc., indicate relatively greater degrees of the bitter taste.

† Juice diluted 1 part juice to 10 parts water before tasting.

be selected for juice extraction, two opposing tendencies must be considered. While the glucoside content decreases with age (**46**), as a general rule the processes of senescence become increasingly important and lead to other undesirable qualities than the bitter taste of the prepared product. The exact range within which the fruit should be selected will depend not only on the variety but also on the local environmental differences which would tend to shift the period in one direction or the other. The rule to be followed in each case would be to harvest the fruit when the glucoside content has decreased sufficiently to reduce to a minimum the tendency of the product to develop a bitter taste and before the processes of senescence have proceeded far enough to bring other undesirable qualities into the product.

EFFECT OF AFTER-TREATMENT OF JUICE ON DEVELOPMENT OF BITTER TASTE.—The vacuumized juice was poured into 8-oz. jelly glasses and again vacuumized during the sealing process. The product was then stored in two ways, at 32° F. (0° C.) without preliminary freezing and at 0° F. (−18° C.) with preliminary freezing. Samples were then removed at convenient intervals for investigation. The first treatment was employed to test the possibility of preserving the juices for short periods, up to 36 hours, in order to meet the demand of the producer who wishes to market unfrozen citrus juices. The second treatment was intended to meet the demand for a feasible method of storing the product over a period up to eight months or longer.

In the case of unfrozen juice stored at 32° F. (0° C.) for short periods of time, satisfactory results have been obtained with methods 1 and 2, so far as controlling the bitter taste is concerned (table IX). In some instances, however, other undesirable taste qualities have given some trouble; therefore method 2 was feasible with these exceptions. With methods 3 and 4, as a general rule the development of the bitter taste could not be effectively controlled even by immediate storage, except in cases where the glucoside was present in minimal quantity. As shown in table IX, the rate and degree of development of the bitter taste were not consistently affected by the treatment. In this connection it should be pointed out that some of the undesirable taste, especially when present in minor quantities, was not readily distinguished when the product was consumed in the cold condition. This indicates that the undesirable taste would likely be less pronounced in actual commercial practice if the product were produced and marketed under low-temperature conditions. It would be necessary to indicate to the consumer, however, that the product might deteriorate rapidly at ordinary room temperatures.

When citrus juices were frozen and stored at 0° F. (−18° C.), partially satisfactory results were obtained in all cases by the use of methods 1 and 2. Valencia orange and Tahiti lime juices put up by method 2 developed no

bitter taste even after four months of storage at 10° (−12° C.) in the frozen condition. Lime juice prepared according to method 4, however, after four months' storage developed a perceptibly bitter taste within an hour after defrosting. The results secured by storing juices in the frozen condition at 0° F. (−18° C.) for shorter periods in the case of various types of citrus, including Satsuma orange, sweet orange, tangerine orange, and lime, were similar to those heretofore reported for Valencia orange and Tahiti lime over a longer period.

OTHER TASTE QUALITIES.—Of the various other taste qualities, desirable and otherwise, only that imparted by the addition of citrus oil is taken up in this report. A minimum addition of citrus-peel oil to the freshly extracted juice is not objectionable and in fact is usually preferred. While the presence of a small amount of oil has not had any bad effect on juice frozen by certain methods, it might result in undesirable taste qualities under some other methods of freezing and storage. After four months' storage, Valencia orange and Tahiti lime juice extracted by method 2 and frozen and stored under vacuum had only the very slightest off-taste, although there was considerable oil content. As pointed out in the preceding discussion, a minimum amount of citrus oil may entirely mask the bitter taste developed in juice prepared by some methods of manufacture.

CHANGES IN COLOR

Second in importance to taste is the color of prepared citrus juices. The orange or yellow coloring matter of the flesh of various citrus types is localized in small bodies (plastids) found in the cells which compose the juice sacs of the fruit (**26**). The plastids vary in shape with the type of citrus.

The red, orange, and yellow pigments occurring in plants fall into several chemical classes: carotinoids, flavones (anthoxanthones), and anthocyanins (**19, 31, 43, 44**). The presence of the carotinoids has been demonstrated in certain citrus juices by MATLACK (**27**). The color changes in citrus juices were studied on two types of material, empty juice sacs, and the juice containing the extracted plastid pigments.

Color descriptions are based on the MAERZ and PAUL color dictionary (**25**). Two types of material were described as to color. In one case the pigments were in solution and in the other the colored particles were suspended in the juice mixture. An 8-oz. tumbler was filled to a depth of 0.75 inch, and the mask was placed over the top of the tumbler which was set on a 600-cc. beaker inverted over a white background. This allowed light to penetrate from all sides, making the optical conditions comparable with those of a liquid in a bottle. When the color was in solution it was not practicable to use this method, and then color comparisons were made by examination of the liquid in the tumbler from the side without a mask. In report-

TABLE X

SOLUBILITY TESTS ON PIGMENTS OF EMPTY JUICE SACS OF VARIOUS CITRUS TYPES

Solvent group*	Solvent	Satsuma (Owari)				Sweet orange (Hamlin)				Tangerine (Dancy)			
		Solubility in 50 cc. solvent	Original color of sacs (10 gm.)	Color of sacs after treatment	Color of filtrate	Solubility in 50 cc. solvent	Original color of sacs (10 gm.)	Color of sacs after treatment	Color of filtrate	Solubility in 50 cc. solvent	Original color of sacs (10 gm.)	Color of sacs after treatment	Color of filtrate
I	Ethyl alcohol 95 per cent.	++	9–9–L† (Deep orange)	9–5–K (Yellow)	9–2–K (Yellow)	+	9–4–L (L. orange yellow)	9–2–K (Yellow)	9–1–I (Yellow)	++	9–9–I (Orange)	9–6–L (Orange)	9–4–K (Orange yellow)
	Acetone	+	9–9–L (Deep orange)	9–3–K (Yellow)	9–2–K (Yellow)	+	9–4–L (L. orange yellow)	9–1–I (Yellow)	9–1–L (Yellow)	++	9–9–I (Orange)	9–5–K (Orange yellow)	9–2–K (Yellow)
	Acetaldehyde	+	9–9–L (Deep orange)	9–4–L (L. orange yellow)	9–2–J (Yellow)	+–	9–4–L (L. orange yellow)	9–1–L (Yellow)	10–1–C (Pale yellow)	++	9–9–I (Orange)	9–5–K (Orange yellow)	9–2–K (Yellow)
	Ethyl ether	+	9–9–L (Deep orange)	9–5–K (Orange yellow)	9–2–J (Yellow)	+–	9–4–L (L. orange yellow)	9–2–K (Yellow)	10–1–C (Pale yellow)	+	9–9–I (Orange)	9–2–K (Orange yellow)	9–2–K (Yellow)
	Chloroform	+	9–9–L (Deep orange)	9–4–L (L. orange yellow)	9–2–J (Yellow)	+–	9–4–L (L. orange yellow)	9–2–K (Yellow)	10–1–C (Pale yellow)	++	9–9–I (Orange)	9–4–L (L. orange yellow)	9–2–K (Yellow)
II	Petrol ether	+–	9–9–L (Deep orange)	9–4–L (L. orange yellow)	9–1–J (Yellow)	–	9–4–L (L. orange yellow)	9–2–K (Yellow)	Colorless	+–	9–9–I (Orange)	9–3–K (Yellow)	10–1–I (Yellow)
III	Benzene	+	9–9–L (Deep orange)	9–4–L (L. orange yellow)	9–3–K (Yellow)	+– –	9–4–L (L. orange yellow)	9–2–K (Yellow)	10–1–C (Pale yellow)	+	9–9–I (Orange)	9–4–L (L. orange yellow)	9–2–J (Yellow)
	Xylene	+	9–9–L (Deep orange)	9–4–L (L. orange yellow)	9–3–K (Yellow)	+– –	9–4–L (L. orange yellow)	9–2–K (Yellow)	10–1–B (Very dim yellow)	+	9–9–I (Orange)	9–4–L (L. orange yellow)	9–3–K (Yellow)
	Toluene	+	9–9–L (Deep orange)	9–4–L (L. orange yellow)	9–3–K (Yellow)	+– –	9–4–L (L. orange yellow)	9–2–L (Yellow)	10–1–B (Very dim yellow)	+	9–9–I (Orange)	9–4–L (L. orange yellow)	9–2–J (Yellow)
	Carbon disulfide	+	9–9–L (Deep orange)	9–8–I (Orange)	9–6–I (Light orange)	+– –	9–4–L (L. orange yellow)	9–4–K (L. orange yellow)	9–2–B (Very dim orange)	+–	9–9–I (Orange)	9–8–J (Orange)	9–5–E (Pale orange)

* Group I dissolves all pigments equally.
Group II dissolves carotin and traces of others.
Group III acts intermediately between first and second groups.

† MAERZ and PAUL number.

ing the color reactions, the general range of color change is indicated in parentheses below the common names.

NATURE OF JUICE-SAC PLASTID PIGMENTS.—The solubility tests made gave results in harmony with the earlier work of MATLACK on the nature of the plastid pigments in citrus fruits (**27**). The data covering the solubility tests with the usual carotinoid solvents are presented in table X. The results of the solubility tests for anthocyanins are given in table XI.

TABLE XI

SOLUBILITY OF FRESH PLASTID PIGMENTS OF VARIOUS CITRUS TYPES IN HOT WATER

TYPE OF CITRUS FRUIT	SOLUBILITY OF ORIGINAL PIGMENT IN 100 GM. OF EMPTY JUICE SACS		SOLUBILITY OF LIGHTER COLORED PIGMENT RESULTING IN JUICE-SAC TISSUE TO WHICH PEEL OIL HAS BEEN ADDED
Satsuma (Owari)	Solubility in 50 cc. solvent	–*	–
	Original color of sacs	9–9–L (Deep orange	9–4–L (Light orange yellow)
	Color of sacs after treatment	9–9–L (Deep orange)	9–4–L (Light orange yellow)
	Color of filtrate	Colorless	Colorless
Orange (Hamlin)	Solubility in 50 cc. solvent	–	–
	Original color of sacs	9–4–L (L. orange yellow)	9–1–L (Yellow)
	Color of sacs after treatment	9–4–L (L. orange yellow)	9–1–L (Yellow)
	Color of filtrate	Colorless	Colorless
Tangerine (Dancy)	Solubility in 50 cc. solvent	–	–
	Original color of sacs	9–9–I (Orange)	9–5–L (Orange yellow)
	Color of sacs after treatment	9–9–I (Orange)	9–5–L (Orange yellow)
	Color of filtrate	Colorless	Colorless
Lime (Key)	Solubility in 50 cc. solvent	–	–
	Original color of sacs	17–2–G (Green)	17–1–J (Light yellow)
	Color of sacs after treatment	17–2–G (Green)	17–1–J (Yellow)
	Color of filtrate	Colorless	(Colorless)

* (–) indicates absence of any particular pigment.

The data reported in table X, using juice-sac material from Owari Satsuma orange, Hamlin sweet orange, and Dancy tangerine, with the usual carotinoid solvents, show that the pigments contained in this material were markedly soluble in ethyl alcohol, acetone, acetaldehyde, and chloroform,

Group II dissolves carotin and traces of others.
Group III acts intermediately between first and second groups.
† MAERZ and PAUL number.

the solvents which, according to TSWETT (**40**), dissolve all the carotinoids equally. The solubility with petrol ether is as a rule slightly less or nil. According to TSWETT, petrol ether mainly dissolves carotin. The third group of solvents used consisted of those acting in an intermediate manner between the foregoing (**40**), benzene, xylene, toluene, and carbon disulphide. These tests as a rule showed marked solubility except in the case of sweet orange. With sweet orange the juice was very light in color, owing to the comparative immaturity of the fruit; with riper fruit a more definite reaction would probably be obtained with these solvents.

The solubility tests with the usual technique used for anthocyanins (**30**) show that the juice-sac pigments of Owari Satsuma orange, Hamlin sweet orange, Dancy tangerine, and Key lime were insoluble in hot water, indicating that anthocyanins are apparently absent.

COLOR CHANGES DUE TO CITRUS OIL AND OTHER COMPOUNDS.—As indicated in table I, the possible effect of citrus oil on the juices prepared by method no. 2 was to change the original Satsuma orange color of the juice to yellow. This opened up the field of color changes in prepared juices. Since no very marked color changes were observed with methods 1, 3, and 4, it seemed logical to look for the cause of the color change in the outer peel which introduced the chief variation in method 2. It was noticed also that the original Satsuma orange color was changed to yellow as a result of neutralization and pasteurization of the juice mixture. The results of a typical experiment covering the effect of citrus oil, neutralization, and pasteurization on color changes in Owari Satsuma orange, Hamlin sweet orange, Dancy tangerine, Key lime, and McCarty grapefruit are presented in table XII. It will be noted that the original color of prepared juices, orange or yellow as the case may be, can readily be changed from orange to a lighter shade of orange, or to yellow, or from yellow to a lighter shade of yellow, by the three methods indicated. In the case of neutralization the color change is reversible within certain limits.

Apparently the source of the citrus peel oil, as shown in table XIII, does not affect the results, since the change takes place in all citrus types studied whether sweet orange, Satsuma orange, tangerine, grapefruit, or lime oil is used. It should be noted also that hand-pressed oil gives the same results as distilled oil. In the sap expressed from sweet orange peel from which the oil had been extracted, as shown in table XII, no characteristic change of color was observed in Satsuma, tangerine, and sweet orange juices. This indicated that the causal agent was present in the citrus oil and not in the rest of the outer peel tissue.

The data in table XIII show also that the degree of observed color change was, within certain limits (1, 2, and 10 parts per 1000 parts of juice), in a marked degree proportional to the amount of citrus oil added. The

TABLE XII

COLOR CHANGES IN CITRUS FRUIT JUICES PRODUCED BY CITRUS OIL, ALKALI, AND PASTEURIZATION

TYPE OF CITRUS FRUIT	ORIGINAL COLOR		AFTER ADDITION OF 2 CC. OF DISTILLED CITRUS PEEL OIL TO 25 CC. OF JUICE		AFTER NEUTRALIZATION WITH 0.1 N NaOH		AFTER PASTEURIZATION AT 180° F. FOR 30 MINUTES	
	MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME
Satsuma orange (Owari)	9–7–L	Deep chrome (orange)	9–1–L	Sulphur (yellow)	9–2–K	Chrome lemon (yellow)	9–4–L	Sunflower (light orange yellow)
Sweet orange (Hamlin)	9–2–I	Pinard (light orange yellow)	9–1–L	Sulphur (yellow)	11–1–K	Acacia (dull green yellow)	9–1–J	Deep Martius (yellow)
Tangerine (Dancy)	9–6–K	Golden glow (orange)	9–1½–L	Sulphur (yellow)	9–3–K	Empire (yellow)	9–4–K	Jasmine (yellow)
Key lime	17–2–G	Sea foam (yellow green)	17–1–J	Martius (light yellow)	17–1½–K	Light citronelle (yellow green)	17–1–J	Martius (light yellow)
Grapefruit (McCarty)	10–1–D	Marguerite (pale yellow)	10–1–F	Light straw (light yellow)	11–1–K	Acacia (dull green yellow)	10–1–F	Light straw (light yellow)

TABLE XIII

EFFECT OF VARYING AMOUNTS OF CITRUS PEEL

ADDED TO 25 CC. JUICE FROM JUICE SACS ONLY	SATSUMA (OWARI) JUICE						
	ORIGINAL COLOR		OBSERVED COLOR			ORIGINAL COLOR	
	MAERZ & PAUL NUMBER	COMMON NAME	TIME INTERVAL	MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME
			hr.				
No treatment (check)	9–7–L	Deep chrome (orange)	¼	9–7–L			
			½	9–7–L			
			3	9–7–L	Deep chrome (orange)	9–6–K	Forsythia (orange)
Distilled orange oil 0.025 cc.			¼	9–6–L			
	9–7–L	Deep chrome (orange)	½	9–4–L			
			3	9–2–L	Chrome lemon (yellow)	9–6–K	Forsythia (orange)
Distilled orange oil 0.05 cc.	9–7–L	Deep chrome (orange)	¼	9–4–L			
			½	9–3–L			
			3	9–1½–L	Chrome lemon (yellow)	9–6–K	Forsythia (orange)
Distilled orange oil 0.25 cc.		Deep chrome (orange)	¼	9–2–L			
			½	9–1–L			
			3	9–1–L	Deep sulphur (yellow)	9–6–K	Forsythia (orange)
Distilled tangerine oil 0.025 cc.			¼	9–6–L			
	9–7–L	Deep chrome (orange)	½	9–4–L			
			3	9–2–L	Chrome lemon (yellow)	9–6–K	Forsythia (orange)
Distilled tangerine oil 0.05 cc.			¼	9–4–L			
	9–7–L	Deep chrome (orange)	½	9–2½–L			
			3	9–1½–L	Chrome lemon (yellow)	9–6–K	Forsythia (orange)
Distilled tangerine oil 0.25 cc.			¼	9–2–L			
	9–7–L	Deep chrome (orange)	½	9–1–L			
			3	9–1–L	Deep sulphur (yellow)	9–6–K	Forsythia (orange)
Distilled grapefruit oil 0.025 cc.			¼	9–6–L			
	9–7–L	Deep chrome (orange)	½	9–4–L			
			3	9–2–L	Chrome lemon (yellow)	9–6–K	Forsythia (orange)
Distilled grapefruit oil 0.05 cc.			¼	9–3–L			
	9–7–L	Deep chrome (orange)	½	9–2½–L			
			3	9–1½–L	Chrome lemon (yellow)	9–6–K	Forsythia (orange)
Distilled grapefruit oil 0.25 cc.			¼	9–2–L			
	9–7–L	Deep chrome (orange)	½	9–1–L			
			3	9–1–L	Deep sulphur (yellow)	9–6–K	Forsythia (orange)
Hand-pressed sweet orange oil 0.25 cc.			¼	9–2–L			
	9–7–L	Deep chrome (orange)	½	9–1–L			
			3	9–1–L	Deep sulphur (yellow)	9–6–K	Forsythia (orange)
Hand-pressed Satsuma oil 0.25 cc.			¼	9–2–L			
	9–7–L	Deep chrome (orange)	½	9–1–L			
			3	9–1–L	Deep sulphur (yellow)	9–6–K	Forsythia (orange)
Hand-pressed tangerine oil 0.25 cc.			¼	9–3–L			
	9–7–L	Deep chrome (orange)	½	9–2–L			
			3	9–1–L	Deep sulphur (yellow)	9–6–K	Forsythia (orange)
Hand-pressed lime oil 0.25 cc.			¼	9–3–L			
	9–7–L	Deep chrome (orange)	½	9–2–L			
			3	9–1–L	Deep sulphur (yellow)	9–6–K	Forsythia (orange)
Orange peel sap less oil 1 cc.			¼	9–7–L			
	9–7–L	Deep chrome (orange)	½	9–7–L			
			3	9–7–L	Deep chrome (orange)	9–6–K	Forsythia (orange)

TABLE XIII

OIL ON COLOR CHANGES IN CITRUS JUICES

Tangerine (Dancy) juice			Sweet orange (Hamlin) juice				
Observed color			Original color		Observed color		
Time interval	Maerz & Paul number	Common name	Maerz & Paul number	Common name	Time interval	Maerz & Paul number	Common name
¼	9–6–K				¼	9–2–I	
½	9–6–K		9–2–I	Pinard (light	½	9–2–I	
3	9–6–K	Forsythia (orange)		orange yellow)	3	9–2–I	Pinard (light orange yellow)
¼	9–5–K				¼	9–1–J	
½	9–4–K		9–2–I	Pinard (light	½	9–1–K	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–K	Sulphur (yellow)
¼	9–4–K				¼	9–1–J½	
½	9–3–K½		9–2–I	Pinard (light	½	9–1–K	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–K½	Sulphur (yellow)
¼	9–2–L				¼	9–1–K	
½	9–2–L		9–2–I	Pinard (light	½	9–1–K½	
3	9–1–I	Deep sulphur (yellow)		orange yellow)	3	9–1–L	Deep sulphur (yellow)
¼	9–5–K				¼	9–1–J	
½	9–4–K		9–2–I	Pinard (light	½	9–1–J½	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–K	Sulphur (yellow)
¼	9–4–K½				¼	9–1–J½	
½	9–3–K½		9–2–I	Pinard (light	½	9–1–K	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–K	Sulphur (yellow)
¼	9–3–L				¼	9–1–K	
½	9–2–L		9–2–I	Pinard (light	½	9–1–K½	
3	9–1–L	Deep sulphur (yellow)		orange yellow)	3	9–1–L	Deep sulphur (yellow)
¼	9–5–K				¼	9–1–J	
½	9–3–K		9–2–I	Pinard (light	½	9–1–J½	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–K	Sulphur (yellow)
¼	9–4–K				¼	9–1–J½	
½	9–3–L		9–2–I	Pinard (light	½	9–1–K	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–K½	Sulphur (yellow)
¼	9–3–L				¼	9–1–K	
½	9–2–L		9–2–I	Pinard (light	½	9–1–L	
3	9–1–L	Deep sulphur (yellow)		orange yellow)	3	9–1–L	Deep sulphur (yellow)
¼	9–3–K				¼	9–1–K½	
½	9–3–K		9–2–I	Pinard (light	½	9–1–L	
3	9–2–J	Chrome lemon (yellow)		orange yellow)	3	9–1–L	Deep sulphur (yellow)
¼	9–3½–L				¼	9–1–K½	
½	9–3–L		9–2–I	Pinard (light	½	9–1–L	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–L	Deep sulphur (yellow)
¼	9–3–L				¼	9–1–K	
½	9–3–L		9–2–I	Pinard (light	½	9–1–L	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–L	Deep sulphur (yellow)
¼	9–3–L				¼	9–1–K	
½	9–3–L		9–2–I	Pinard (light	½	9–1–L	
3	9–2–L	Chrome lemon (yellow)		orange yellow)	3	9–1–L	Deep sulphur (yellow)
¼	9–6–K				¼	9–2–I	
½	9–6–K		9–2–I	Pinard (light	½	9–2–I	
3	9–6–K	Forsythia (orange)		orange yellow)	3	9–2–I	Pinard (light orange yellow)

TABLE XIV

EFFECT OF VARIOUS OILS ON COLOR CHANGES IN EMPTY JUICE SACS OF CITRUS TYPES

TREATMENT	CLASSIFICATION	SATSUMA ORANGE (OWARI)		TANGERINE (DANCY)		SWEET ORANGE (HAMLIN)	
		ORIGINAL COLOR	OBSERVED COLOR	ORIGINAL COLOR	OBSERVED COLOR	ORIGINAL COLOR	OBSERVED COLOR
Orange oil	Essential oil	9–9–L* Sunkiss† (deep orange)	9–4–L Sunflower (light orange yellow)	9–9–J Mikado (orange)	9–5–L Apricot (orange yellow)	9–4–L Sunflower (light orange yellow)	9–1–L Sulphur (yellow)
Clove oil	Essential oil	9–9–L Sunkiss (deep orange)	9–5–K Apricot (orange yellow)	9–9–J Mikado (orange)	9–5–L Apricot (orange yellow)	9–4–L Sunflower (light orange yellow)	9–1–L Sulphur (yellow)
Cedar oil	Essential oil	9–9–L Sunkiss (deep orange)	9–4–L Sunflower (light orange yellow)	9–9–J Mikado (orange)	9–5–L Apricot (orange yellow)	9–4–L Sunflower (light orange yellow)	9–1–L Sulphur (yellow)
Citronella oil	Essential oil	9–9–L Sunkiss (deep orange)	9–4–L Sunflower (light orange yellow)	9–9–J Mikado (orange)	9–5–L Apricot (orange yellow)	9–4–L Sunflower (light orange yellow)	9–1–L Sulphur (yellow)
Turpentine oil	Essential oil	9–9–L Sunkiss (deep orange)	9–4–L Sunflower (light orange yellow)	9–9–J Mikado (orange)	9–5–L Apricot (orange yellow)	9–4–L Sunflower (light orange yellow)	9–1–I Sulphur (yellow)
Canada balsam	Resin	9–9–L Sunkiss (deep orange)	9–4–L Sunflower (light orange yellow)	9–9–J Mikado (orange)	9–5–L Apricot (orange yellow)	9–4–L Sunflower (light orange yellow)	9–1–L Sulphur (yellow)
Avocado oil	Fruit and vegetable oil	9–9–L Sunkiss (deep orange)	9–7–L Deep chrome (orange)	9–9–J Mikado (orange)	9–8–K Capucine (orange)	9–4–L Sunflower (light orange yellow)	9–4–L Sunflower (light orange yellow)

TABLE XIV—*Continued*

Treatment	Classification	Satsuma orange (Owari)		Tangerine (Dancy)		Sweet orange (Hamlin)	
		Original color	Observed color	Original color	Observed color	Original color	Observed color
Pecan oil	Vegetable oil	9–9–L Sunkiss (deep orange)	9–8–L Cadmium (orange)	9–9–J Mikado (orange)	9–8–J Genista (orange)	9–4–L Sunflower (light orange yellow)	9–4–L Sunflower (light orange yellow)
Castor oil	Vegetable oil	9–9–L Sunkiss (deep orange)	9–8–L Cadmium (orange)	9–9–J Mikado (orange)	9–8–J Genista (orange)	9–4–L Sunflower (light orange yellow)	9–4–L Sunflower (light orange yellow)
Linseed oil	Vegetable oil	9–9–L Sunkiss (deep orange)	9–8–L Cadmium (orange)	9–9–J Mikado (orange)	9–8–J Genista (orange)	9–4–L Sunflower (light orange yellow)	9–4–L Sunflower (light orange yellow)
Glycerol	Alcoholic constituent of non-volatile fats and oils	9–9–L Sunkiss (deep orange)	9–8–L Cadmium (orange)	9–9–J Mikado (orange)	9–9–J Mikado (orange)	9–4–L Sunflower (light orange yellow)	9–4–L Sunflower (light orange yellow)
Cod-liver oil	Marine animal oil	9–9–L Sunkiss (deep orange)	9–7–L Deep chrome (orange)	9–9–J Mikado (orange)	9–8–L Cadmium (orange)	9–4–L Sunflower (light orange yellow)	9–4–L Sunflower (light orange yellow)

* Maerz and Paul number.
† Common name

figures show also that within these limits the rate of color change was in a marked degree proportional to the amount of oil added.

The experiment was carried a step further in a preliminary attempt to determine the factors involved. The data are shown in table XIV. The following groups of oils and resins and glycerol were added to citrus juice pressed from juice sacs only (Satsuma, tangerine, and sweet orange): (a) essential oils—orange oil (check treatment), clove oil, cedar oil, citronella oil, turpentine; (b) resin—Canada balsam; (c) fruit and vegetable oils—avocado oil, pecan oil, castor oil, linseed oil; (d) cod-liver oil; and (e) alcoholic constituent of non-volatile fats and oils—glycerol. The results were fairly consistent in showing that the essential oils and resins have a similar effect in changing the color of citrus-juice mixtures, as has the check treatment with orange oil, which is also of the same group. The fruit and vegetable oils, cod-liver oil, and the glycerol, had no effect in changing the color except a very slight one in the case of Satsuma orange juice mixture.

In table XV preliminary data are presented showing some changes in color of prepared citrus juices due to the method of extraction and after-treatment, by freezing and cold storage. The characteristic color changes due to small amounts of citrus oil present in the citrus-juice mixture have been discussed. The data presented in table XV are concerned primarily with the effect of freezing and cold storage on the color of the prepared product. In general, it should be noted that in practically all cases there is a slight change in color because of these treatments. The data recorded show that the change was from orange with a medium yellow tone to the same color with a lighter yellow tone. In only a few exceptional cases was there a change in the opposite direction, changes from darker or lighter shades of the same color, or *vice versa*, simultaneous with a change in tone value (see discussion in MAERZ and PAUL **25**).

In considering the application of these results, it should be realized that in citrus juices with a naturally deep Satsuma, tangerine, or orange color, any changes to lighter shades of the same color, or to a yellow color, would be a distinct drawback; however, in the case of dull shades of yellow, a change to a brighter yellow may constitute an improvement.

Consideration of the relative amounts of citrus oil required to obtain a given color effect is of importance, not only to those choosing a method of preparation already available, but also to those interested in the design of improved machinery for juice preparation.

As indicated at the beginning of this section, the color factor is secondary to that of taste, and the amount of citrus oil used must in every case be considered from the standpoint of agreeableness in taste of the product.

TABLE XV

EFFECT OF METHOD OF EXTRACTION AND TREATMENT ON COLOR CHANGES IN CITRUS JUICES

VARIETY	DATE	METHOD OF PREPARATION	AFTER-TREATMENT	STORAGE PERIOD	COLOR	
					MAERZ & PAUL NUMBER	COMMON NAME
				days		
Sweet orange (Hamlin)	11/20/31	1	Original juice	0	9–1–J	Bright Martius (yellow)
"	"	1	Frozen	5	9–1–I	Martius (yellow)
"	"	2	Original juice	0	9–2–L	Chrome lemon (yellow)
"	"	2	Cold stored	3	9–1–K	Light sulphur (yellow)
"	"	2	Frozen	5	9–1–L	Sulphur (yellow)
"	"	3	Original juice	0	9–3–L	Bright empire (yellow)
"	"	3	Cold stored	3	9–1–K	Light sulphur (yellow)
"	"	3	Frozen	5	9–1½–L	Sulphur (yellow)
"	"	4	Original juice	0	9–4–L	Sunflower (yellow)
"	"	4	Cold stored	3	9–1½–L	Sulphur (yellow)
"	"	4	Frozen	5	9–1½–L	Sulphur (yellow)
Sweet orange (Pineapple)	11/23/31	1	Original juice	0	9–1½–J	Pinard (yellow)
"	"	2	Original juice	0	9–2–L	Chrome lemon (yellow)
"	"	2	Cold stored	2	9–1½–L	Sulphur (yellow)
"	"	2	Frozen	2	9–1½–L	Sulphur (yellow)
"	"	3	Original juice	0	9–2–L	Chrome lemon (yellow)
"	"	3	Cold stored	2	9–1½–L	Sulphur (yellow)
"	"	3	Frozen	2	9–1½–L	Sulphur (yellow)
"	"	4	Original juice	0	9–2–L	Chrome lemon (yellow)
"	"	4	Cold stored	2	9–1½–L	Sulphur (yellow)
"	"	4	Frozen	2	9–1½–L	Sulphur (yellow)
Satsuma (Owari)	11/19/31	1	Original juice	0	9–8–L	Cadmium (deep orange)
"	"	1	Frozen	6	9–6½–L	Golden glow (orange)
"	"	2	Original juice	0	9–9–L	Sunkiss (deep orange)
"	"	2	Cold stored	1	9–8½–L	Cadmium (deep orange)
"	"	2	Cold stored	4	9–8½–L	Cadmium (deep orange)
"	"	2	Cold stored	6	9–8–L	Cadmium (deep orange)
"	"	2	Frozen	6	9–8–L	Cadmium (deep orange)
"	"	3	Original juice	0	9–10–K	Marigold (deep orange)
"	"	3	Cold stored	1	9–9–L	Sunkiss (deep orange)
"	"	3	Cold stored	4	9–8½–L	Cadmium (deep orange)
"	"	3	Cold stored	6	9–8–L	Cadmium (deep orange)
"	"	3	Frozen	6	9–8–L	Cadmium (deep orange)
"	"	4	Original juice	0	9–10–L	Marigold (deep orange)
"	"	4	Cold stored	1	9–9–L	Sunkiss (deep orange)
"	"	4	Cold stored	4	9–8–L	Cadmium (deep orange)
"	"	4	Cold stored	6	9–8–L	Cadmium (deep orange)
"	"	4	Frozen	6	9–8–L	Cadmium (deep orange)
Tangerine (Dancy)	11/23/31	1	Original juice	0	11–3–E	Light maple (dull orange yellow)
"	"	2	Original juice	0	9–7–L	Deep chrome (orange)
"	"	2	Cold stored	4	9–6½–K	Forsythia (orange)
"	"	2	Frozen	4	9–7½–L	Deep chrome (orange)
"	"	3	Original juice	0	9–8–L	Cadmium (deep orange)
"	"	3	Cold stored	4	9–8–K	Capucine (deep orange)
"	"	3	Frozen	4	9–7½–L	Deep chrome (orange)
"	"	4	Original juice	0	9–8–L	Cadmium (deep orange)
"	"	4	Cold stored	4	9–8–K	Capucine (deep orange)
"	"	4	Frozen	4	9–7½–L	Deep chrome (orange)

CHANGES IN DIRECTION AND RATE OF MOVEMENT OF SUSPENDED PARTICLES

Extracted citrus juices are made up of two distinct phases: one is an almost transparent liquid phase containing mainly dissolved substances, such as sugars, acids, and some colloidal material; the other is a solid phase consisting of small particles of juice sac, locular wall, inner-peel or other tissue in suspension in the liquid phase. The particles in suspension carry the pigment which gives the juice its color, and are closely associated with the characteristic taste of orange juice. Clarified juices do not have this characteristic taste and cannot be substituted for the freshly extracted juice. The larger solid particles begin separating from the liquid phase immediately after extraction. If the juice is allowed to stand long enough undisturbed, there will result an almost purely liquid stratum, which is water clear, and a second stratum or strata of a minimum of liquid and most of the solid particles. In prepared juices this stratification will finally take place, but the methods of preparation and other factors have a pronounced effect upon the *rate* at which it takes place. Other things being equal, the juice showing the slowest rate of stratification is most desirable.

In determining the direction and rate of movement of particles in the juice mixture, the samples were shaken up and poured into 100-cc. graduates, and records of the amount and direction of movement, as well as differences in turbidity in the different parts of the cylinder were taken at 15-min. intervals during the first hour, and at greater intervals for the next 20 hours, and recorded as volume of a stratum.

Only relative differences in turbidity are indicated in the present report. A more accurate nephelometer method (**22, 23**) is at present under consideration. According to the method used, the turbidity of the sample was observed in the 100-cc. graduated cylinder. The readings were taken primarily on the less turbid upper portion, or on the central portion when some of the suspended particles had risen. The symbols used, and their relative values, are more or less arbitrary: Thus (++++) represented a condition in which the liquid was practically opaque; increasing degrees of translucency are represented by (+++), (++), (+), and (+−); when the liquid became almost transparent, so that outlines of objects could be seen through the cylinder, the condition is represented by (+ − −); increasing degrees of transparency are indicated by (+−−−), etc.

The prepared citrus-juice mixture, as indicated in the previous discussion, is a buffered biological solution containing in addition extracted solutes and suspended particles derived from the tissues of the fruit during the process of preparation. It was logical, therefore, to look for the causes of changes in the stability of the prepared mixture in the interplay of the factors contained in it and possibly the external environmental conditions. Apparently, therefore, the method of preparation should have a profound

effect upon the total mass in suspension, the direction and rate of its movement up or down, and also the relative turbidity of the liquid mixture. The stage of maturity of the fruit which furnished the juice, and the effect of various subsequent treatments of the juice (such as the containers, gases in contact with the juice, freezing, storage temperature, and length of storage period) are also of importance in this connection.

The exact rôle of the various component parts as they affect the stability of the citrus-juice mixture has not been definitely determined. The constituents possibly affecting the character of the juice mixture may be divided into three classes: (a) substances in true solution, such as sugars, citric acid, proteins, and pectins; (b) substances present in the colloidal state, such as proteins and pectins; (c) matter in mass composed of larger suspended particles.

The first step in the attempt to understand the stability of the suspension was to study the behavior of infusions of the tissues of the fruit in the concentrated condition, also diluted with juice extracted from juice sacs only, and with water. The tissues considered were the outer peel, inner peel and veins, locular walls, central axis, seed coat, and cotyledon and germ. The locular wall, inner peel, and vein infusions gave the most turbid and stable mixtures; the germ and cotyledon infusions also gave turbid and stable infusions. When tissue infusions were diluted in the proportions of 2, 4, and 8 parts of infusion to 100 parts of juice from juice sacs only, or with water, the results paralleled those already indicated, showing that even small amounts of these tissue infusions have a marked effect on the stability of the suspension as measured by the relative turbidity.

As previously stated, the turbidity and color of the juice mixture are due to particles of tissue introduced by the method of extraction, and these tend to separate out from the liquid phase. The direction and rate of movement may be affected by the size and specific gravity of the particles in suspension, or by the possible presence or absence of stabilizing substances such as oils, pectins, and proteins or precipitants (electrolytic or colloidal), or by indirect effects such as might be produced by enzymes. A preliminary report will be given here on the effect of the size of the particles, of citrus oil, and of cold storage and freezing.

The effect of decreasing the size of the particles on the stability of the suspension was studied by using a ball mill and a colloid mill to reduce the size of the particles. In general, the stability of the suspension increases with decrease in size of particles, as indicated by the number of hours the material was ground in a ball mill (table XVI), while the color intensity decreases with decrease in the size of the particles. It will be noted also that the turbidity of the translucent portion is directly correlated with the increase of stability, as indicated by (+−−) turbidity at the end of three hours

TABLE XVI

EFFECT ON STABILITY OF CITRUS-JUICE SUSPENSION OF DECREASING SIZE OF PARTICLES BY BALL-MILL GRINDING

VARIETY	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	ZONE	VOLUME IN CC. OF ZONE OR ZONES CONTAINING SEPARATED SOLIDS AFTER VARYING PERIODS OF TIME* (HOURS)										
					¼	½	¾	1	2	3	4	5	6	8	24
Tangerine (Dancy) Bartow	11–24–31	2	Untreated	Top	.....	.....	.....	.....	20	18	.....	16	14	18	8
				Bottom	.....	.....	.....	.....	7	6	.....	20	20	20	7
				Turbidity‡	.....	.....	.....	.....	.....	.....	.....	.....	.....	+–	+–––
"	"	2	Ball mill 1 hour	Top	6	5	5	5	.....	5	3	3	3	.....	0
				Bottom sand†	0	30	26	24	.....	14	14	14	15	.....	7
				Bottom tissue	.....	0	0	0	.....	5	4	4	5	.....	7
				Turbidity‡	.....	.....	++	++	.....	++	++	++	++	.....	++
"	"	2	Ball mill 2 hours	Top	No top zone formed										
				Bottom sand†	.....	.....	.....	.....	10	10	8	7	.....	.....	5
				Bottom tissue	.....	.....	.....	.....	0	0	0	1	.....	.....	1
				Turbidity‡	.....	.....	.....	.....	+++	+++	+++	+++	.....	.....	+++
Tangerine (Dancy) Hawthorne	"	2	Untreated	Top	.....	.....	.....	.....	57	46	39	35	.....	.....	29
				Bottom	.....	.....	.....	.....	0	0	0	8	.....	.....	11
				Turbidity‡	.....	.....	.....	.....	.....	+––	+––	+––	.....	.....	+–––
"	"	2	Ball mill 2 hours	Top	No top zone formed										
				Bottom sand†	.....	.....	.....	19	17	14	.....	.....	.....	.....	5
				Bottom tissue	.....	.....	.....	0	0	0	.....	.....	.....	.....	4
				Turbidity‡	.....	.....	.....	.....	++	++	.....	.....	.....	.....	++
															16 hr.
"	"	2	Ball mill	Top											0
				Bottom sand†											3
				Bottom tissue											4
				Turbidity‡											+++

* Volumes read in 100-cc. cylinders.

† In grinding a liquid in a ball mill, some sandy or powdery rock material is produced. When juice ground in this way is set up in cylinders, a heavy precipitate, chiefly composed of ground-up rock, comes down quickly and is followed later by settlings that are derived from material in the juice; these zones are separately reported here. The sand zone contained a small proportion of citrus tissue.

‡ Turbidity symbols as indicated in text.

TABLE XVII

EFFECT OF VACUUMIZATION ON STABILITY OF CITRUS-JUICE SUSPENSION

VARIETY AND SOURCE	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	ZONE	VOLUME IN CC. OF ZONE OR ZONES CONTAINING SEPARATED SOLIDS AFTER VARYING PERIODS OF TIME* (HOURS)										
					¼	½	¾	1	2	3	4	5	6	8	24
Dancy tangerine (Bartow)	11–24–31	2	Untreated	Top	.....	.....	.....	.....	20	18	.....	16	14	18	8
				Bottom	.....	.....	.....	.....	7	6	.....	20	20	20	17
				Turbidity†	.....	.....	.....	.....	.....	.....	.....	.....	.....	+–	+–––
Dancy tangerine (Hawthorne)	11–24–31	2	"	Top	.....	.....	.....	.....	57	46	39	35	.....	.....	29
				Bottom					0	0	0	8	.....	.....	11
				Turbidity†	.....	.....	.....	.....	.....	+––	+––	+––	.....	.....	+–––
"	"	2	Juice vacuumized	Top	0										
				Bottom	97	80	.....	.....	.....	58	55	52	.....	.....	38
				Turbidity†	.....	.....	.....	.....	.....	+–	+–	+–	.....	.....	+–––
"	"	2	"	Top	0										
				Bottom	.....	95	92	.....	80	26	.....	.....	.....	.....	54
				Turbidity†	.....	.....	+–	.....	+–	+–	.....	.....	.....	.....	+–––

* Volumes read in 100-cc. cylinders.

† Turbidity symbols as indicated in text.

for Dancy tangerines in untreated material and (+ +) for a similar sample ground in the ball mill for two hours. A second experiment was carried out with juices run through a colloid mill. The results to some extent parallel those secured by the use of the ball mill, although the colloid mill was not as effective in this regard as the ball mill. As was to be expected, the color of the juice mixture changed to a lighter shade with a decrease in the size of the particles. The results obtained by the use of a high-speed soda fountain stirrer were very good, giving a more stable suspension than was obtained by use of the colloid mill.

As has been pointed out earlier, the juices were all subjected to vacuum treatment in order to remove the dissolved gases, a variable factor that would have been brought in if the material had not been treated in this manner. In general, when untreated citrus juices were allowed to stand at room temperature after thorough shaking, they separated into three strata. A small portion of the particles moved upward and a larger portion settled at the bottom, leaving a more or less translucent stratum between (table XVII). When the juices were vacuumized, however, the tendency was for all the particles to move downward, leaving a more or less translucent upper stratum.

The rates of settling during the first few hours in juices prepared by methods 1 and 2 were as a rule similar to the rate in those prepared by method 4, this being the slowest. In the case of method 1, a few larger particles settled out quickly, but the turbidity of the remainder of the juice was so great as to mask this. When the turbidity is greatly reduced for some reason, this layer of coarse particles becomes apparent and the rate of settling would be very rapid if this were taken as the indication of settling. The rate of settling for juices prepared by method 3 is much more rapid than for methods 1, 2, and 4. For method 2 there is little or no settling during the first hour or so after standing. For method 3 there is a rapid settling during the same period, and in method 4 the tendency is again similar to that for method 2. After standing for more than two or three hours, the rate of settling for methods 2 and 4 apparently proceeds at a rate similar to that of method 3; and after twenty hours the amount of settling is approximately the same for methods 2 and 3, but for method 4 the absolute value is higher within the period indicated (table XVIII).

After the juices prepared by the four methods were put in glass containers and vacuumized, two methods of further treatment were employed,—cold storage at 32° F., and freezing at −20° to −25° F. (−28° to −31° C.) with storage in the frozen condition at 0° F. Some of the experimental results concerning the possible effects of the two methods of storage on the stability of the juice mixture are summarized in tables XVIII and XIX.

TABLE XVIII

EFFECT OF METHOD OF EXTRACTION AND AFTER-TREATMENT ON STABILITY OF CITRUS-JUICE SUSPENSION

TYPE OF FRUIT AND VARIETY	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	STORAGE PERIOD	VOLUME IN CC. OF MORE TURBID REGION AND TURBIDITIES* OF LESS TURBID REGION AFTER VARYING PERIODS OF TIME (HOURS)							
					¼	½	¾	1	2	3	4	24
				days								
Orange (Hamlin)	11/20	1	Original juice		.....	.....	.....	.....	.....	8	.....	.....
"	"	1	Frozen	5	6	.....	6	.....	5	5	5	5
					++		++		+	+	+	++
"	"	2	Original juice		.....	96			70		48	37
"	"	2	Cold stored	3	96	94	78	71	61	56	52	.....
"	"	2	Frozen	5	95	.....	94	.....	81	72	63	37
					+		+		+−	+−−	+−	+−
"	"	3	Original juice		.....	65	.....	.....	.....	53	.....	39
"	"	3	Cold stored	3	98	97	90	84	74	67	63	.....
"	"	3	Frozen	5	74	.....	55	.....	43	40	39	30
					+		+		+	+−	+−	+
"	"	4	Original juice		99	98	97	97	97	.....	.....	79
"	"	4	Cold stored	3	98	98	97	97	95	94	92	.....
"	"	4	Frozen	5	.....	.....	95	.....	90	85	81	50
							+		+	+	+	+
Satsuma (Owari)	11/19	1	Original juice		.....	12	.....	.....	10	.....	8	.....
"	"	1	Frozen	6	.....	.....	6	.....	6	6	6	6
							++		++	++	++	++
"	"	2	Original juice		.....	97	.....	93	.....	.....	.....	45
"	"	2	Cold stored	1	98	.....	.....	.....	86	60	.....	44
"	"	2	Cold stored	4	97	95	90	85	77	70	65	.....
									++	++	+++	.....
"	"	2	Cold stored	6	.....	93	91	85	80	70	65	42
							++	+	+	+	+−	.....
"	"	2	Frozen	6	95	.....	88	.....	68	63	60	40
					+		+		+	+	+	+
"	"	3	Original juice		.....	85	.....	68	.....	.....	.....	33
"	"	3	Cold stored	1	96	.....	.....	.....	69	52	.....	37
"	"	3	Cold stored	4	92	86	73	67	59	55	51	.....
"	"	3	Cold stored	6	.....	80	71	64	61	54	52	37
							++	+	+	+	+−	+−
"	"	3	Frozen	6	63	.....	46	.....	39	32	30	23
					+		+		+	+	+	+
"	"	4	Original juice		.....	93	.....	75	63	.....	.....	.....
"	"	4	Cold stored	1	98	.....	.....	.....	83	62	.....	40
"	"	4	Cold stored	4	98	96	94	91	88	83	78	.....
									+++	+++	+++	.....
"	"	4	Cold stored	6	.....	90	86	83	80	70	66	42
							++	++	++	++	++	++
"	"	4	Frozen	6	72	.....	59	.....	46	42	39	29
					+		+		+	+	+	+
Tangerine (Dancy)	11/23	1	Original juice		.....	95	.....	.....	.....	.....	.....	27
"	"	2	Original juice		.....	.....	.....	96	.....	.....	.....	63
"	"	2	Cold stored	4	100	98	98	97	97	.....	89	51
						+	+		+		+−	+−−

* Turbidity symbols as indicated in text.

TABLE XVIII (*Continued*)

Type of fruit and variety	Date prepared	Method of preparation	After-treatment	Storage period	Volume in cc. of more turbid region and turbidities* of less turbid region after varying periods of time (hours)							
					¼	½	¾	1	2	3	4	24
				days								
Tangerine (Dancy)	11/23	2	Frozen	4	94	84	76	70	.....	46	.....	29
					+	+−	+−	+−		+−−		+−−
"	"	3	Original juice		.....	.....	.....	98	95	92	.....	61
"	"	3	Cold stored	4	100	98	97	95	94	.....	72	45
						+	+−	+−	+−		++	−−
"	"	3	Frozen	4	94	84	76	70	.....	46	.....	29
					+	+−	+−	+−		+−−		+−−
"	"	4	Original juice		.....	.....	.....	99	97	94	.....	80
"	"	4	Cold stored	4	100	99	99	99	99	95	.....	70
						+	+−	+−	+−	+−	+−	−−
"	"	4	Frozen	4	100	97	98	90	.....	.....	.....	59
						+−	+−	+−				+−
Lime (Tahiti)	7/22	2	Frozen	4 mo.	37	30	.....	25	21	20	19	15
					++	++		++	++	++	++	++
"	"	4	Frozen	4 mo.	40	34	.....	29	25	23	23	15
					+++	++		++	++	++	++	++

* Turbidity symbols as indicated in text.

In table XIX data are presented showing that after four months of storage (juice prepared by method 2 and the rate of freezing varying from 25 minutes to 12 hours) the treatment gave the following results: with the slower rates of freezing, 10 and 12 hours, the settling in general was more rapid than with the quicker rates, 25 to 110 minutes.

The data presented in table XVIII concerning the effect on the rate of settling when juices prepared by the four methods are stored at 32° F., or in the frozen condition at 0°, show with few exceptions that the rate of settling is not markedly affected by these after-treatments.

In certain methods of manufacture, citrus oil is introduced into the juice. An experiment was carried out to determine the effect of citrus oil on the relative stability of the suspension. When a small amount of citrus oil was added to the citrus juice, a portion of the particles were found to rise to the top, as shown in table XX. Other experiments showed that when excessive amounts of citrus oil were added, all of the larger suspended particles in the juice mixture rose to the top.

Summary

1. The method of localizing in the tissues of the citrus fruit whenever possible the cause or causes of undesirable qualities in the prepared citrus-juice mixture has yielded important results.

TABLE XIX

EFFECT OF RATE OF FREEZING ON STABILITY OF CITRUS-JUICE SUSPENSION (VALENCIA SWEET ORANGE)

VARIETY	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	STORAGE PERIOD	TIME REQUIRED FOR FREEZING	VOLUME IN CC.* OF ZONE OR ZONES CONTAINING SEPARATED SOLIDS AFTER VARYING PERIODS OF TIME (HOURS) AND TURBIDITY† OF SUPERNATED LIQUID								
						¼	½	¾	2	3	4	6	8	24
Sweet orange (Valencia)	7/24/31	2	Frozen	*months* 4	25 minutes	100	33	27	21	20	19	17	15	15
						++	+++	+	+	+	+	+	+	+
"	"	2	Frozen	4	30 minutes	62	48	38	30	27	25	24	22	20
						++	++	+	+	+	+	+	+	+
"	"	2	Frozen	4	90 minutes	100	44	32	35	23	21	20	20	18
						++	++	+	+	+	+	+	+	+
"	"	2	Frozen	4	110 minutes	80	68	53	39	34	31	28	25	23
						++	++	+	+	+	+	+	+	+
"	"	2	Frozen	4	10 hours	60	44	35	27	25	24	21	21	20
						++	++	+	+	+	+	+	+	+
					12 hours	65	49	38	29	27	25	22	22	20
						++	++	+	+	+	+	+	+	+

* Volumes read in 100-cc. cylinder.
† Turbidity symbols as indicated in text.

TABLE XX

EFFECT OF PRESENCE OF CITRUS OIL ON STABILITY OF CITRUS-JUICE SUSPENSION

VARIETY	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	ZONE	VOLUME OF SEPARATED ZONE IN CC. AND TURBIDITY‡ AFTER VARYING PERIODS OF TIME (HOURS)							
					¼	½	¾	3½	6	9	11	24
Orange (Hamlin)	12–16–31	2 (Vacuumized)	Untreated	Bottom	0	95	84	60	50	45	43	35
				Top			+−−	+−−	+−−−	+−−−	+−−−	+−−−
				Bottom			+++	+++	+++	+++	+++	+++
"	"	2 (Vacuumized)	1 per cent. citrus oil added	Top*	90		Breaking	17	15	15	15	10
				Center*			Curdy	45	40	40	35	34
				Bottom*				10	10	10	10	10
				Translucent				+−−	+−−	+−−−	+−−−	+−−−
				Separated†				+++	+++	+++	+++	+++
Tangerine (Dancy) ...	"	2 (Vacuumized)	Untreated	Bottom	0	.....	85	43	32	29	25	22
				Top				+−	+−	+−	+−	+−
				Bottom				+++	+++	+++	+++	+++
"	"	2 (Vacuumized)	1 per cent. citrus oil added	Top*	0	.....	Curds	35	30	30	30	20
				Bottom*				10	12	12	12	10
				Top				++++	++++	++++	++++	++++
				Center				++	+	+	+	−−
				Bottom				+++	+++	+++	+++	++++

* When oil was added to orange juice, all of the solids started to rise and then became curdy and separated out to form an upper, a central, and a bottom layer, with two zones of translucent liquid separating them; the volume rather than the exact position of the central zone is shown.

† Since the two translucent zones appeared identical and the turbidity of the zones containing separated solids also appeared identical, the turbidities are given under these two headings.

‡ Turbidity symbols as indicated in text.

2. The cause of the bitter taste which develops in prepared citrus juice on aging has been identified as of glucosidal origin and has been localized primarily in the inner-peel, veins, and locular wall tissues, the degree of its development being a function of fruit maturity, citrus type and variety, method of preparation, and after-treatment.

3. The kind of bitter taste developed for the samples tested was found to depend on the nature of the glucoside contained in the particular type of citrus fruit.

4. The mechanism involved in the development of a bitter taste in the prepared citrus-juice mixture was found to be non-enzymatic in nature.

5. The tendency of prepared citrus juices to develop a bitter taste on aging was found to decrease with maturity of the fruit used. This is in harmony with the known fact that the glucoside content of citrus fruits decreases with maturity.

6. Minimum amounts of citrus-peel oil for the period of time covered by the experiments did not prove objectionable from the standpoint of taste quality.

7. Undesirable taste qualities other than the distinctly bitter taste were encountered under certain conditions. Certain of these must be effectively controlled before a completely satisfactory frozen or cold-stored product can be secured.

8. The causal agent for the observed color changes in prepared citrus juices was traced to the outer peel and identified as citrus oil. When citrus oil was added to the juice mixture the orange or yellow color was changed to brighter shades of the same color, or to yellow, or from yellow to lighter shades of yellow. Somewhat similar results were obtained by neutralizing and by pasteurizing the juice mixture as well as by the addition of some other essential oils and resins.

9. Within the limits of the experiments, the amount and the rate of change in color were in a marked degree proportional to the quantity of citrus oil added.

10. The stability of the suspension is being investigated on the basis of the constituents brought into the juice mixture by the method of manufacture.

11. Reducing the size of the suspended particles by grinding or other treatments improved the stability of the suspension and lightened the color of the product.

12. In prepared citrus juices a portion of the suspended particles rose and another portion settled; following vacuumization all of the suspended particles settled.

13. The method of preparation had a material effect upon the rate of movement of the suspended particles immediately after thorough mixture

with the juice; but after two to four hours' standing the rates of movement of the particles were similar for all methods of preparation used.

14. Preliminary experiments seemed to indicate that rates of freezing had a greater effect upon the stability of the suspension than types of storage. Quick freezing and cold storage of juice did not materially affect the rate of movement of the suspended particles as compared with freshly prepared juice.

15. The presence of small quantities of citrus oil caused a portion of the particles to rise instead of settle and very large amounts caused all particles to rise.

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SIEVE-TUBE STRUCTURE AND TRANSLOCATION IN THE POTATO

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(WITH PLATES II–VII AND ONE FIGURE)

Introduction

The movement of carbohydrates into the potato tuber lends itself to quantitative study. The products of assimilation in the leaves move through the stems and stolon and are condensed to starch within storage cells in the tuber. The stolon may be readily sectioned for measurement; and, knowing the transverse area of its phloem and the rate of development of the tuber, one can calculate the rate of translocation during growth. Photosynthesis is lacking in underground structures, and respiration may be estimated with a fair degree of accuracy.

BIRCH-HIRSCHFELD (**3**) used a similar method to measure transportation from the leaf of *Phaseolus multiflorus;* and DIXON (**10**) calculated a rate of 50 cm. per hour for flow of a 10 per cent. sucrose solution through the phloem of a potato stolon during a 100-day growth period. In a previous paper (**4**) the writer arrived at a somewhat lower rate, 21 cm. per hour, for flow through the total phloem area of the potato stolon during the period of most rapid growth. As respiration losses were not accounted for, this value represents merely the lower limit; actual rates are undoubtedly greater. Many translocation rates have been reported in the literature within the last decade; and, although differing individually, they all approximate this order of magnitude.

Ringing experiments, from the early ones of MALPIGHI (**18**), HALES (**12**), and KNIGHT (**17**) to those more recently performed by CURTIS (**6, 7, 8**), MASON and MASKELL (**19, 20**), and SCHUMACHER (**28**), have indicated that organic nutrients synthesized in the leaves move downward through the phloem tissues of the stem. The writer has proposed (**4**) that the osmotic system described by MÜNCH (**22**) may provide the force necessary to cause this downward flow; and he has attempted to describe a mechanism (**4, 5**) which will fit the quantitative data on translocation and at the same time conform to the anatomy of the plant.

Experiments described by SCHUMACHER indicate that the sieve tubes play an important part in the movement of organic materials out of the leaf. As perforation of the sieve plates has probably no essential rôle in longitudinal movement (**5**), we need some other explanation for the relation of the sieve tube to translocation. A previous paper (**5**) cited evi-

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dence that the permeability of the sieve tubes in cucurbits increases with maturity. The present work discusses the detailed anatomy of sieve tubes in the potato in relation to translocation, and provides data on rates of flow by which the general theory may be further tested.

Phloem anatomy

Although ARTSCHWAGER (**1, 2**) has studied the gross anatomy of the potato, the details of sieve-tube structure have not been described. Because a knowledge of the developmental phases of sieve-tube anatomy must underlie the interpretation of their physiology in relation to translocation, several of these features are included in the present study.

The general vascular structure of the potato resembles that of many herbaceous plants. The bundles are bicollateral. In the stem they tend to remain discrete, being connected laterally by interfascicular cambium; but they fuse more or less in the root and stolon, forming a cylinder of irregular thickness. The phloem is confined to the vascular bundles more strictly than in the cucurbits, but the internal strands follow devious paths and form an anastomosing network in the central region of the stem (**1,** pl. 34–35). Although anastomosis of the phloem strands is also found in the external phloem, sieve tubes do not occur outside the endodermis or starch sheath; nor do the structural differences noted in the cucurbits (**5**) appear.

Sieve tubes are perceptible in very early stages in sections of young stolons cut from imbedded material. At the time the sieve-tube mother cell divides to form sieve tube and companion cell, or soon afterwards, the slime body appears in the cytoplasm. ARTSCHWAGER has described and illustrated these bodies (**2,** pl. 1*D*, 6, 7), showing one of them in a sieve-tube element, the nucleus of which was still in the metaphase (**2,** pl. 6*B*). In the potato these structures differ considerably during their early stages from the slime drops of the cucurbits, only one appearing in each sieve-tube element. In their later development, however, they pass through similar stages, disintegrating as the sieve tube matures.

Plate II, figure 1, shows two of these structures in two successive sieve-tube elements of the young potato stolon. The nuclei are still present, protoplasmic strands have not appeared in the sieve plate, and plasmolysis is apparent in the upper element. Figure 2 shows a slime body in a short connecting element and figure 3 illustrates a later stage. While the slime body and nucleus are present the protoplast seems normal, accumulating neutral red within the vacuole and becoming plasmolyzed in hypertonic solutions. As the element approaches its mature size, however, the nucleus enlarges and disintegrates (fig. 4); the slime body gradually expands, becomes fibrous in structure (plate III, figs. 5, 6), and slowly spreads out

into a tangled mass of gelatinous strands (figs. 7, 8, 9). At this stage, streaming slows down to a plastic flow, and the internal protoplasmic meshwork described in cucurbits (**5**) is formed (figs. 9, 10). The tangled mass of disintegrating slime may become attached to these moving protoplasmic strands and presents a peculiar writhing motion.

Small leucoplasts imbedded in the protoplasm, and up to this time practically indistinguishable, begin to accumulate condensed carbohydrate, forming spheres (plate III, fig. 9, plate IV, fig. 16, and plate V, fig. 19) which stain pink or violet with iodine. During the early stages of sieve-tube development, while neutral red is readily accumulated, the vacuoles of these cells are distinct and easily distinguished from the parietal cytoplasmic layer. As the nucleus and slime body disintegrate and the leucoplasts enlarge, the phase boundary between vacuole and cytoplasm apparently breaks down; the plastids migrate from the periphery to the central region of the cell, becoming violently agitated by Brownian movement; and neutral red fails to accumulate within the cell. Slime plug formation, which up to this time has not been noted, is readily induced by means of alcohol and other killing agents. In young tissues (plate III, figs. 6, 8, 10, and plate IV, figs. 11, 12) slime plugs are made up of conglomerate masses of partially disintegrated slime bodies and nuclei, but as disintegration becomes complete they are formed of the more homogeneous suspension that results (plate IV, figs. 13, 14, 15).

The protoplasmic layer of the sieve tube reacts differently to different reagents. The condition depicted in figure 14 results from the use of a killing agent composed of three parts of absolute alcohol to one part of glacial acetic acid. That in figure 15 was induced by 95 per cent. alcohol, which causes a severe shrinking of the protoplasm. The internal strands are present, however, and may be densely stained by the use of iodine and anilin blue (plate IV, figs. 13, 16, and plate V, fig. 17). In figure 13 the slime, which is small in volume, has formed on the surface of the sieve plate a compact layer through which the protoplasmic strands project.

By rapidly killing thin sections of potato stolon tissue in this alcohol-acetic acid mixture, one can avoid contraction and staining of the protoplasm; while the suspension of colloidal material within the cell is coagulated to a flocculent reticulum distributed uniformly throughout the cell (plate V, figs. 18, 19). The protoplasm of these mature sieve tubes shows peculiar properties in fresh sections. All streaming movements have ceased, neutral red no longer accumulates, the cells cannot be plasmolyzed, and anilin blue penetrates readily, staining the callus of the sieve plates (fig. 20) and fragments of colloidal material within the cell. Plate VI, figures 21 and 22 illustrate this stage. The former shows a longitudinal section of phloem of potato stolon that has been allowed to accumulate neutral

red for some time. As soon as this picture was taken, a hypertonic sucrose solution was applied under the cover glass; and, as the cells containing neutral red became plasmolyzed, the photograph shown in figure 22 was made. While the sieve tube in the center of these figures was unaffected, the companion cells and several phloem parenchyma cells were severely plasmolyzed.

Although the protoplasm seems to have passed into an inert condition, it is not actually dead, for treatment with alcohol will often cause a radical change or entire cessation of Brownian movement of the plastids; and killing with dilute eosin solution by the method of SCHUMACHER (**28**) will cause rapid callusing of the sieve plates and collapse of the elements. Apparently a condition is reached where distinctions between protoplasm and vacuole become less pronounced; the internal strands and parietal layer are simply portions having greater density or viscosity than the intervening regions.

As the sieve tubes become senile, the plates are heavily callused (fig. 20), as are the side wall pits; and in some instances large spherical masses of callus may partially or almost completely fill the cell lumina. Similar callus formations may be induced by the use of dilute eosin solutions (**28**), and after their development the tubes soon collapse and become obliterated.

The sieve plates of potato develop in the same way as those of cucurbits (**5**), the chief differences being that the protoplasmic connections are smaller, the callus cylinders are less prominent, and definitive callus formation is less pronounced. The optical effect resulting from the focusing of light by the callus cylinders is very striking in potato sieve plates. Figures 23 and 24 show pictures of the same sieve plate taken at slightly different levels, a water immersion lens and a 12× ocular being used. Apparently these plates show an interference phenomenon, the light being blotted out in figure 23 and reinforced in figure 24, for these white spots are much brighter than the original unoccupied field of the microscope.

An oil immersion lens reveals structures similar to those found in cucurbits. The crater-like ends of developing callus cylinders appear in plate VII, figure 25. When stained heavily with anilin blue after being mordanted in iodine, the protoplasmic strands appear as small black dots surrounded by blue callus cylinders (**15**), which in turn lie in an almost unstained cellulose matrix. The differentiation is difficult to photograph because the differences in color are greater than those in intensity and because one can seldom find plates which are clear of adhering protoplasm and flat enough to show more than a few strands in focus at one time. Figure 26 shows a cup-shaped sieve plate with the edge in focus, giving a longitudinal view of the protoplasmic strands; and figure 27 shows them in transverse view. Although the photographs are not very satisfactory, the

actual preparations are quite convincing and confirm the interpretation that these minute dark-staining cores are the actual protoplasmic strands. As they are only 0.3–0.5 μ in diameter, pores within these strands, if such were present, could probably not be detected by means of the microscope.

The slime bodies in potato sieve tubes have only a short existence. During the major portion of their functioning period, the sieve tubes are devoid of these bodies and of nuclei; the limits of the central vacuoles cannot be detected in fresh sections; a great number of starch-containing plastids are suspended within the confines of the parietal layer; and the protoplasm, by its staining and plasmolytic properties, seems to have lost almost completely its property of semipermeability. The companion cells have nuclei and are rather densely filled with protoplasm, while the phloem parenchyma cells are nucleate and contain starch in increasing quantities as they mature.

Translocation studies

As detailed anatomical study seems to render untenable the classical theory of mass flow through open pores in the sieve tubes, we must search critically for another mechanism to explain translocation. As in previous work (**4, 5**), dissection studies have been made on the stems and stolons of the potato.

Phloem exudation is not so pronounced as in the cucurbits, for several reasons. As soon as an incision is made into the phloem, the intercellular spaces of the adjacent parenchyma tissues become filled with sap, acquiring a water-soaked appearance. If another cut is then made near the first, sap will be seen to flow out and wet the surface of the stem with a very thin film that spreads rapidly. These two observations show that the exuded sap has a very low surface tension, differing from that of cucurbits.

If more cuts are made in quick succession near the first one, sap will be seen to accumulate rapidly and run from the wound. If then a cut is made through into the xylem, the sap will be drawn in rapidly and the wounded area will become dry. This shows, as do other experiments, that the phloem exudate for the potato does not coagulate in the same way as cucurbit sap. In addition, one should remember that exudation in the cucurbit stem starts at a rate from three to eleven times that of normal flow (**5**), while in the potato the stem is apparently less elastic, so that little more than the normal rate is observed.

From measurements of the tubers and stolons of potatoes grown in the field, more reliable rates of translocation may be calculated. The plants were of the Russet Rural variety and were grown by the Division of Vegetable Crops of the College of Agriculture, Cornell University, Ithaca, New York.

TABLE I

MEASUREMENTS ON TUBERS AND STOLONS OF POTATO

No.	Tuber			Stolon					
					Area of transverse section of				
	Fresh wt.	Dry wt.	Dry wt. as % of fresh wt.	Length	Stolon	External phloem	Internal phloem $cm.^2 \times 10^{-2}$	Total phloem	Cell wall phloem area ratio
	gm.	*gm.*	*per cent.*	*cm.*	*cm.²*	*cm.²*		*cm.²*	*per cent.*
1	353.0	83.6	23.7	2.0	0.0867	1.98	0.31	2.29	29.7
2	163.0	34.5	21.2	1.8	0.0472	0.63	0.30	0.93	30.2
3	181.5	38.7	21.4	2.3	0.0934	1.28	0.46	1.74	32.7
4	139.5	31.8	22.8	1.7	0.0937	0.93	0.62	1.55	30.7
5	87.0	16.4	18.9	1.7	0.0580	0.83	0.41	1.24	30.2
6	237.6	54.4	22.9	1.4	0.1011	1.03	0.57	1.60	28.6
7	164.5	36.0	21.9	0.5	0.0527	0.63	0.25	0.88	32.4
8	117.2	25.8	22.0	1.3	0.0678	0.85	0.36	1.21	30.1
9	74.2	17.3	23.4	0.8	0.0583	0.60	0.35	0.95	37.8
10	164.3	39.0	23.8	0.9	0.0885	1.11	0.52	1.63	40.1
11	136.0	33.4	24.6	2.2	0.0442	0.55	0.32	0.87	34.2
12	126.6	29.0	22.9	0.8	0.0918	1.11	0.69	1.80	35.6
13	108.2	25.4	23.5	0.2	0.1397	0.90	0.69	1.59	34.5
14	100.5	22.5	22.4	1.3	0.0658	0.97	0.32	1.29	30.7
15	477.2	102.5	21.5	0.8	0.3900	4.21	3.17	7.37	31.1
16	101.8	23.2	22.8	0.3	0.0917	1.52	0.67	2.19	32.5
17	112.5	26.7	23.7	1.8	0.0424	0.47	0.30	0.77	36.6
Total	2,844.6	640.2	.....	21.8	1.6130	19.60	10.31	29.91	.....
Average	165.0	37.6	22.5	1.3	0.0950	1.15	0.61	1.76	32.5

The seed pieces were planted on May 17, and the tubers were dug on September 22, 1930. Weights and phloem areas are given in table I.

The measurements of sieve-tube elements of both inner and outer phloem appear in table II, together with sieve-tube area : phloem area ratios. The latter are somewhat higher than the ratio given in a previous calculation (**4**), while the cell walls occupy a somewhat smaller proportion of the total phloem area.

TABLE II

MEASUREMENTS OF SIEVE-TUBE ELEMENTS IN FIVE POTATO STOLONS

STOLON	EXTERNAL PHLOEM		INTERNAL PHLOEM		$\frac{\text{SIEVE-TUBE AREA}}{\text{PHLOEM AREA}} \times 100$
	NUMBER OF ELEMENTS MEASURED	AVERAGE LENGTH	NUMBER OF ELEMENTS MEASURED	AVERAGE LENGTH	
		mm.		*mm.*	
1	18	0.114	12	0.123	21.3
2	11	0.117	8	'0.109	22.4
3	16	0.159	44	0.108	20.1
4	43	0.107	40	0.088	25.6
5	20	0.096	17	0.082	25.2
Average of all	.....	0.106	.....	.....	22.9

Measurements were made on sieve plates from these stolons, the protoplasmic strands counted, and the sieve-plate areas surrounding the strands computed, as shown in table III.

The average sieve-plate area surrounding each strand was 3.04×10^{-8} cm.^{2} The protoplasmic strands were stained and measured in fully hydrated sections; they averaged 0.3 μ in diameter. The area of the transverse section of each strand was 0.0707 μ^2, and the percentage of the sieve-plate area occupied by strands was $0.0707 \div 3.04 \times 100$, or 2.3 per cent.

Data on the rate of growth of Russet Rural potatoes under field conditions at Ithaca were provided by Professor E. V. HARDENBURG of the Vegetable Crops Division. The potatoes were planted on June 10, and separate lots harvested on August 25, September 15, and October 17. Table IV shows that between the first two harvests the tubers gained on an average 173.0–90.3, or 82.7 gm. This gain in 21 days gives a rate of gain of 3.94 gm. fresh weight per day. According to the dry weight composition given in table I, the daily increment of dry weight was 0.89 gm.

These tables provide a basis for comparing, in potato, the different theories of translocation. The total phloem area in the stolons of the seventeen tubers, as shown in table I, averaged 0.0176 cm.^{2} In testing the

TABLE III

AREAS, NUMBER OF STRANDS, AND AREA PER STRAND VALUE FOR SIEVE PLATES OF POTATO

SIEVE PLATE	AREA $CM.^2 \times 10^{-6}$	NUMBER OF STRANDS	AREA ÷ NO. OF STRANDS $CM.^2 \times 10^{-3}$
1	3.39	134	2.53
2	1.50	68	2.24
3	2.60	101	2.58
4	2.87	105	2.73
5	2.75	111	2.48
6	6.34	201	3.15
7	4.47	158	2.83
8	2.79	58	4.81
9	2.04	34	6.00
10	2.99	56	5.34
11	3.30	67	4.93
12	3.04	103	2.34
13	3.15	69	4.57
14	3.35	63	5.32
15	2.38	46	5.17
16	1.61	61	2.64
17	1.46	67	2.18
18	2.27	99	2.29
19	1.25	53	2.36
20	4.80	121	3.96
21	4.05	108	3.75
22	5.62	151	3.72
23	3.67	130	2.82
24	3.84	133	2.88
25	1.92	110	1.75
Total	77.45	2507	.....
Average	3.10	100.3	3.04

protoplasmic streaming hypothesis, it seems best to calculate the movement on a dry weight basis. The daily increment of 0.89 gm. of dry matter would occupy approximately 0.60 cc. and would have to move in the pure state at a linear rate of $\frac{0.60}{0.0176 \times 24}$, or 1.42 cm. per hour, through the total phloem area. Only about 10 per cent. of the phloem, however, is occupied by streaming protoplasm, one-half of which is flowing in the proper direction; so that if it were carrying with it organic matter equal to its own volume, and were loading and unloading it with machine-like precision, the rate of movement would still have to be 56.8 cm. per hour in order to deliver the required material. But the maximum rate of streaming observed in phloem parenchyma of the potato was 1.8 cm. per hour, while in mature

TABLE IV

GROWTH OF POTATO TUBERS

ROW	PLANTED JUNE 10	HARVESTED	TUBERS PER ROW	YIELD PER ROW	WEIGHT PER TUBER
				gm.	*gm.*
2–7–12	"	Aug. 25	143.5	12,970	90.3
3–8–13	"	Sept. 15	141.0	24,390	173.0
1–6–11	"	Oct. 17	157.3	28,900	184.0

sieve tubes no protoplasmic movements occur in sectioned material. Furthermore, since the length of phloem elements is around 0.1 mm., there would be 100 end walls per centimeter, or, in the average conducting system of 30 cm., about 3000 to be crossed by diffusion. If the total gradient in concentration were from 1 molal to 0, then across any one of these membranes the gradient would be $\frac{M}{3000}$, an insignificant value so far as its quantitative aspect is concerned. Streaming through the protoplasmic strands of the sieve plate would be equally inadequate, because dry matter alone would have to move at a rate of $1.42 \div 0.229 \div 0.023$, or 270 cm. per hour, through the pores occupied by these structures. Even if they were distended by internal pressure, as might be conceivable, they would not suffice, for dry matter alone would have to move 6.2 cm. per hour, or over three times the rate of streaming through the total sieve-tube lumen area. Protoplasmic streaming would seem entirely too slow to act as an effective accelerating or transporting mechanism in the movement of organic nutrients through the potato stolon.

As all of the studies on the structure of sieve plates indicate that the protoplasmic strands traversing them are solid, further testing of the mechanism of mass flow through perforations seems useless. The strands themselves in potato are very slender, being less than 1 μ in diameter; and any pores which could possibly traverse them would necessarily be so small that an immense pressure would be needed to cause the required rate of flow.

When one considers movement through the intermolecular spaces in the hydrated cellulose of which the phloem walls are composed, the pressure again is obviously of an impossibly high order.

Assuming that these intermolecular spaces occur between concentric cylinders (**5**), so that the formula as modified to apply to flow between parallel planes might be used, and, inserting a plausible value for the pressure, the value derived for the minimal distance between the planes is 0.9 μ.

This is from 10 to 100 times too great for pore spaces in a jell. As there is no justification for assuming that the resistance to flow through cell walls is less than that calculated, the original theory (**4**) must be modified.

Evidence presented in the previous section indicates that sieve tubes increase in permeability with age; and, in potato, soon reach a condition in which they fail to accumulate neutral red and cannot be plasmolyzed with hypertonic solutions. Repeated trials with sugar beet and cucurbit phloem have given the same results. These findings are contrary to the generally accepted (**3, 22**) ones of RUHLAND which earlier led the writer to the conclusion that the sieve tubes played no part in the transport of organic nutrients (**4**). Continued study on this point, however, convinces one that RUHLAND was either dealing with very young sieve tubes or observing other phloem elements.

With the sieve-tube protoplasm completely permeable, the cross walls would present the greatest resistance to mass flow. The pressure necessary to cause movement through the lumina would be almost negligible. If the dry weight increment of the tuber entered in the form of a 10 per cent. solution, it would move at a linear rate of $\frac{0.89 \times 9}{0.0176 \times 24} = 19.0$ cm. per hour through a passage equal, in cross-section, to the total phloem. Through the 22.9 per cent. of this area occupied by sieve tubes, it would move at a linear rate of 83.0 cm. per hour, or 0.023 cm. per second, requiring a pressure difference of 0.0035 atmospheres per sq. cm. through the stolon.[2]

A study of the volume relationships of the potato plant indicates that the stolon is a region where the transverse phloem area is constricted. The pressure gradient necessary to cause flow through sieve-tube lumina in the entire plant, therefore, is probably not more than ten times this value, the difference between this and the actual pressure in the phloem being required to overcome the resistance of cross walls.

If the sieve-tube end walls were so arranged as to act as a complete obstruction across the total phloem at frequent intervals, then the area available for longitudinal flow through wall material would be the sum of the sieve-tube lumen area plus the sum of the phloem wall area. An average value for this sum would be, from tables I and II, 55.4 per cent. of the total phloem and the average rate through this area 34.3 cm. per hour. In the potato, however, many of the sieve plates are not transverse and seldom do many of them occur in any one plane. By this arrangement a much

[2] Calculated according to POISEUILLE'S equation, $P = \frac{8 R_l n l}{r^2}$ where P = pressure in dynes per sq. cm.; R_l, linear rate of displacement = 0.023 cm. per sec.; n, viscosity of the solution = 0.012; l, average length of stolon = 1.3 cm.; and r, average sieve-tube radius = 0.0009 cm. $P = \frac{8 \times 0.023 \times 0.012 \times 1.3}{0.81 \times 10^{-6}} = 3543$ dynes or 0.0035 atmospheres.

greater surface is exposed, reducing even more the actual rate of movement through wall material. This is brought out in text figure 1 *A*, showing the strictly transverse arrangement, and *B*, the situation (greatly foreshortened) as it occurs in potato.

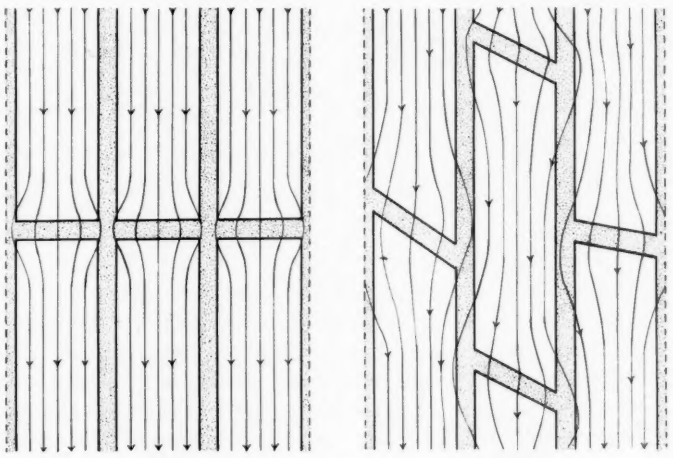

FIG. 1. Diagrammatic representation of flow through potato phloem.

The arrows in these diagrams represent the flow of solution, the distance between them being inversely proportional to the rate of flow. From *B* it can be seen how the actual rate of flow through the wall material may be greatly decreased, reducing the pressure required to maintain movement. By using this reduced rate in the modified formula (**5**) and substituting the volumes and pressures computed for sap concentrations of 10, 15, and 20 per cent., values have been calculated for the minimal distance between parallel planes which would accommodate this flow. As the stolon is a constricted region, a greater pressure gradient per unit length is required. The values are computed on the assumption that 10, 20, or 50 per cent. of the total available pressure is utilized within this limited region.

These values (table V) show that by assuming ideal conditions, namely, a high concentration in the flowing sap and a high utilization of energy within the limited stolon region, one may arrive at dimensions for capillary spaces which approach the probable values. Respiration has not been considered, and a zero pressure is being assumed at the "sink" or storing parenchyma cell. If, on the other hand, hexose sugars and organic acids of low molecular weight were present in the sap, considerably higher pressures might be expected.

TABLE V

DIMENSIONS OF CAPILLARY SPACES REQUIRED FOR CONDUCTION IN POTATO STOLON; CALCULATIONS BASED ON SUCROSE

SOLUTE	CONC.	OSMOTIC PRESSURE AT 25° C.	DIMENSIONS OF CAPILLARY SPACES DISTANCE BETWEEN PARALLEL PLANES		
			PRESSURE UTILIZED		
			10 PER CENT.	20 PER CENT.	50 PER CENT.
per cent.	*molal*	*atm.*	μ	μ	μ
10	0.33	8.5	0.066	0.046	0.029
15	0.52	13.5	0.043	0.031	0.019
20	0.73	19.4	0.033	0.023	0.015

The potato stolon is apparently a very efficient conducting organ; and if a mechanism can be found which will satisfactorily explain its functioning, other cases should present no difficulty. Longitudinal movement through structures lacking mature sieve tubes seems to take place along the walls, and dissection experiments indicate that in growing shoots and root tips this movement is not limited to the phloem but spreads throughout the structure. Apparently those tissue layers which, in the mature stem, limit flow to the phloem, have not yet become functional, the epidermis alone serving to check loss by leakage.

MÜNCH (**22**) has suggested that lateral movement from mesophyll to vascular tissue and from vascular tissue to storage tissues or secondary meristems takes place from living cell to living cell by way of the plasmodesma. In that case, these connecting strands should be abundant in the parenchyma cells of the potato tuber, especially in the vicinity of the phloem strands. Examination of these tissues showed this hypothesis to be correct; and figures 28, 29, and 30 illustrate these structures, stained with gentian violet and iodine. If the plasmodesma are actually wall structures, as JUNGERS (**16**) has recently suggested, it seems strange that when the wall is swelled by the use of 30 per cent. sulphuric acid to double its original thickness (fig. 31), the plasmodesma should undergo no greater change in length than those treated with only 5 per cent. acid (fig. 32).

Upon entering the tuber, the vascular tissue of the stolon multiplies rapidly in amount and the rate of flow is materially reduced. Lateral diffusion from the strands diminishes this rate even more, longitudinal movement becoming relatively slow.

Measurements were made on two tubers; and the resultant values, given in table VI, aid in the determination of these reduced rates.

TABLE VI

MEASUREMENTS ON POTATO TUBERS

TUBER NO.	WEIGHT OF TUBER	LENGTH OF TUBER	TRANSVERSE DIAMETERS OF TUBER	VOLUME OF TUBER	AREA OF TRANSVERSE SECTION	NUMBER OF VASCULAR BUNDLES	AREA OF VASCULAR BUNDLES	AREA OF VASCULAR BUNDLES AS PER CENT. OF TOTAL
	gm.	*cm.*	*cm.*	*cc.*	*cm.*^{2}		*cm.*^{2}	*per cent.*
1	237.0	9.4	7.2 × 6.1	220.3	33.4	827	2.07	6.2
2	242.0	9.7	7.5 × 5.5	225.0	32.5	806	1.73	5.3
Sum	479.0	19.1	.....	445.3	65.9	1633	3.80	.....
Average	239.5	9.5	7.3 × 5.8	222.6	32.9	816	1.90	5.8

As the area occupied by phloem in the stolon is roughly proportional to the weight, for these tubers it would be about $\frac{239.5}{165} \times 0.0176$, or 0.0255 cm.^{2} Dividing the area occupied by phloem in the vascular bundles of the tuber by this value, $1.9 \times 2/3 \div 0.0255$, we find that the phloem expands approximately 50 times within the tuber at its maximum girth. The mean expansion should be about two-thirds this value; and the mean rate of flow within the tuber, if one considers the continual loss by diffusion, should be around 0.285 cm. per hour. The average length of vascular bundle in these tubers is 5.3 cm., and the average time required by the solution to reach its final location within the phloem is $\frac{5.3}{0.285}$, or 18.6 hours.

In calculating the rate at which solute diffuses from the phloem to its final destination within the storage cells, the mean distance must be found by computing the radius of the circle whose area is one-half that of the total area of parenchyma surrounding each vascular strand. Correcting for the area of the bundle itself and for the increase in total area due to growth, the mean distance is 0.047 cm., and the mean rate of movement 0.0025 cm. per hour.

As protoplasmic streaming is common within the parenchyma cells of growing potato tubers, it might possibly explain the movement of solutes from end to end of these cells, leaving only the thickness of walls to be traversed by diffusion. According to measurements, the walls occupy about 3.7 per cent. of the distance. Then $0.047 \times 0.037 = 0.00174$ cm., the distance to be traveled by diffusion. If streaming accounts for an effective movement of 0.018 cm. per hour, the materials would move 96.3 per cent. of the distance in 2.5 hours; 16.1 hours would remain for diffusion; and $0.00174 \div 16.1 = 0.00011$ cm., or 1.1 μ per hour, the rate of diffusion.

This rate proves to be 3.01×10^{-8} cm. per second, or, through unit cross-sectional area, 3.13×10^{-8} gm. per second. If the concentration decreased 0.1 molal within the 0.047 cm., the diffusion constant would be 5.5×10^{-10} for movement across the total area, or 0.11×10^{-7} for diffusion through the 5 per cent. occupied by plasmodesma. This value is about 100 times smaller than that for diffusion through water, and closely approximates that obtained by STEWARD (**29**).

MÜNCH has calculated rates of movement in leaf tissues. From the data on potato (**22,** p. 87), according to his method, a rate of 6.25 μ per day for movement within the leaf was obtained. As plasmodesma in the leaf are confined to pits, and as acceleration by streaming would be confined to the parietal layer, the actual rate of movement is probably many times this. If it were as great as 100 times this value, it would be only

625 μ per day, or 26 μ per hour. Dividing the mean distance of diffusion, 62.5 μ, by 26, we find that 2.4 hours would be the average time consumed. Streaming, common again in these tissues, would carry materials 95 per cent. of the way in 0.3 hours; and the remaining distance, 3.125 μ, divided by the remaining time, 2.1 hours, gives a diffusion rate through the plasmodesma of 1.5 μ per hour.

As the values obtained by these various calculations all lie within the realm of possibility, apparently this combination of diffusion across walls along plasmodesma, acceleration by streaming within living cells, and mass flow through vascular tissues may satisfactorily explain the conduction of organic nutrients in the potato.

Discussion

The studies made on potato and on cucurbits indicate that translocation by diffusion (**26**), protoplasmic streaming (**6, 7, 8, 9**), young sieve tubes (**24**), conducting parenchyma cells (**11, 26**), or perforations through the sieve plates (**23, 22**) can play little part in phloem exudation or in the rapid transport of carbohydrate into the tuber. While the mechanisms of the first two theories seem too slow and the second two are incompatible with present concepts of the living protoplasmic structures of cells, the last, in spite of its classical position, fails because critical study has not demonstrated the presence of open pores in sieve plates.

The errors of the early workers are not difficult to understand in view of the instruments they used. To HARTIG (**13**), NÄGELI (**23**), VON MOHL (**21**), and SACHS (**26**), the sieve plates undoubtedly appeared to be truly perforated, and the phenomenon of phloem exudation seemed to confirm this conclusion. One wonders, however, why no one before SCHMIDT (**27**) pointed out the fallacy of this view, and why his work has aroused so little comment.

SACHS' concept of conducting parenchyma (**26**), although based almost entirely upon circumstantial evidence and although partially refuted by HEINE (**14**), is still accepted by HABERLANDT (**11**) and others.

PRIESTLEY'S idea of movement by streaming through differentiating sieve tubes (**24**) seems difficult to accept because, in many leaves having practically no secondary tissue, translocation continues long after differentiation has ceased, and because, in such structures as the potato stolon, movement would be restricted to an insignificant fraction of the phloem.

Although the evidence for increasing permeability of the sieve tube with maturity is only fragmentary at present, if subsequent studies, using various methods and many plants, confirm this observation, it should prove ex-

tremely important in relation to rapid translocation. No other mechanism can so well explain the limiting cases exemplified in this study.

Summary

1. Because the potato is particularly favorable for translocation studies, the anatomy and physiology of phloem tissues in this plant have been investigated.

2. Young sieve tubes of the potato are nucleate and display the characteristics of normal living cells.

3. As the sieve-tube elements mature, the nuclei and slime bodies disintegrate; and the protoplasm apparently changes its organization, becoming more and more permeable.

4. Slime plugs are shown to be artifacts formed by the action of killing agents upon the vacuolar contents; pores within the protoplasmic strands of the sieve plates could not be demonstrated.

5. Phloem exudate from the potato has a low surface tension, does not coagulate rapidly, and seems to emerge at a rate of flow that would account for normal translocation.

6. Measurements indicate that a 10 per cent. sucrose solution would have to flow 19 cm. per hour through a conduit equal in transverse area to the total phloem to provide for tuber formation.

7. The theories of protoplasmic streaming and pressure flow through phloem walls seem inadequate to explain this rate of movement.

8. Sieve-tube lumina apparently afford the most available channels for this movement, the parietal protoplasm offering little resistance. Capillary spaces of from 0.01 to 0.06 μ would allow movement across end walls under the available pressure.

9. The phloem increases greatly in cross-sectional area within the potato tuber. The rate of flow is correspondingly reduced.

10. Protoplasmic streaming may accelerate lateral movement across non-vascular tissues.

11. Calculations indicate a rate of 1.1 μ per hour for diffusion along plasmodesma of cross walls in the tuber. The corresponding diffusion rate in the leaf would be 1.5 μ per hour.

12. Diffusion along plasmodesma of cross walls and acceleration by protoplasmic streaming within non-vascular tissues, combined with pressure flow through permeable sieve tubes and phloem walls within specialized conducting organs, seem most satisfactorily to explain translocation in the potato.

The writer feels deeply indebted to Dr. O. F. Curtis and other members of the Department of Botany at Cornell University for their aid and interest in this work. He also wishes to thank those members of the Col-

lege of Agriculture at Davis, California, who have helped in the preparation of the manuscript.

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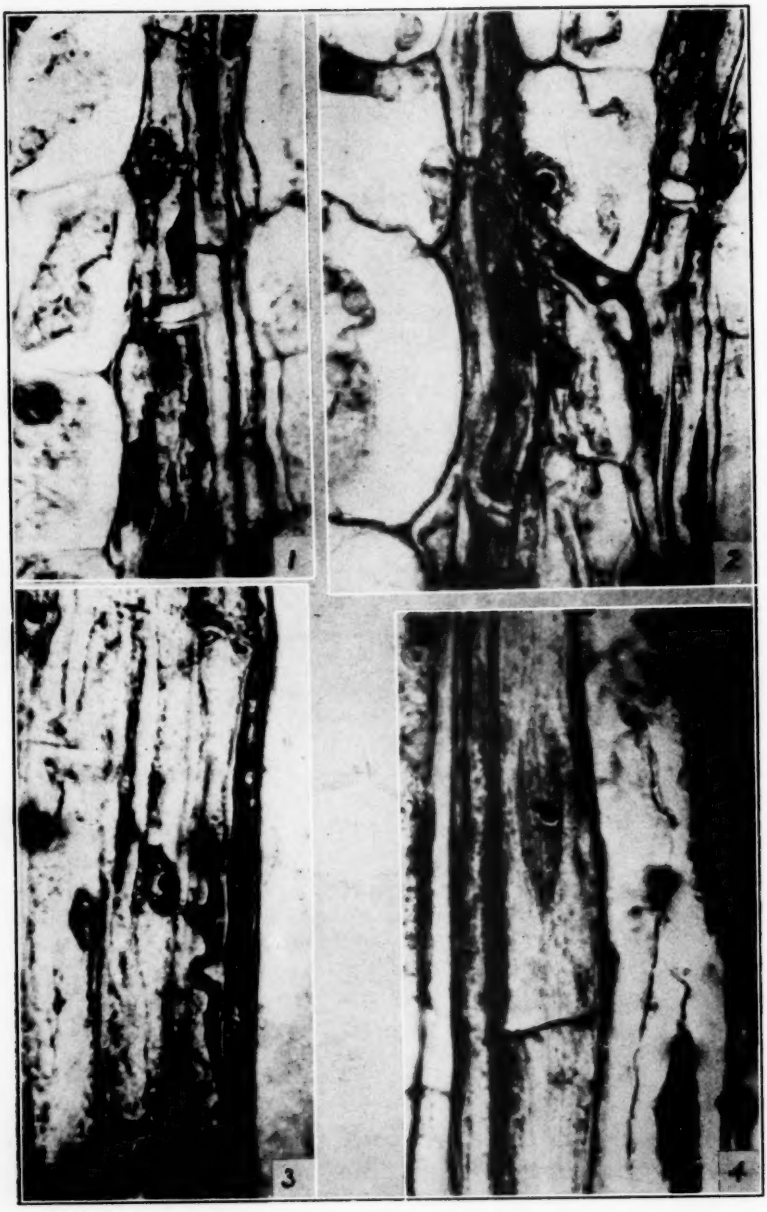

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Explanation of Plates

PLATE II

Fig. 1. Longitudinal section of phloem strand in young potato stolon, showing sieve-tube elements soon after division of sieve-tube mother cell; imbedded material stained with haematoxylin and light green.

Fig. 2. Slime body in short sieve-tube segment of anastomosing internal phloem strands of young potato stolon; same stain as for fig. 1.

Fig. 3. Slime body in developing sieve-tube segment. Note nucleus and densely staining contents of companion cell; same stain as for fig. 1.

Fig. 4. Sieve-tube element in older stolon; nucleus is disintegrating, being enlarged and only lightly stained; staining like fig. 1. All $\times 750$.

PLATE III

FIG. 5. Disintegrating slime body, showing gelatinous strands starting to separate. ×1200.

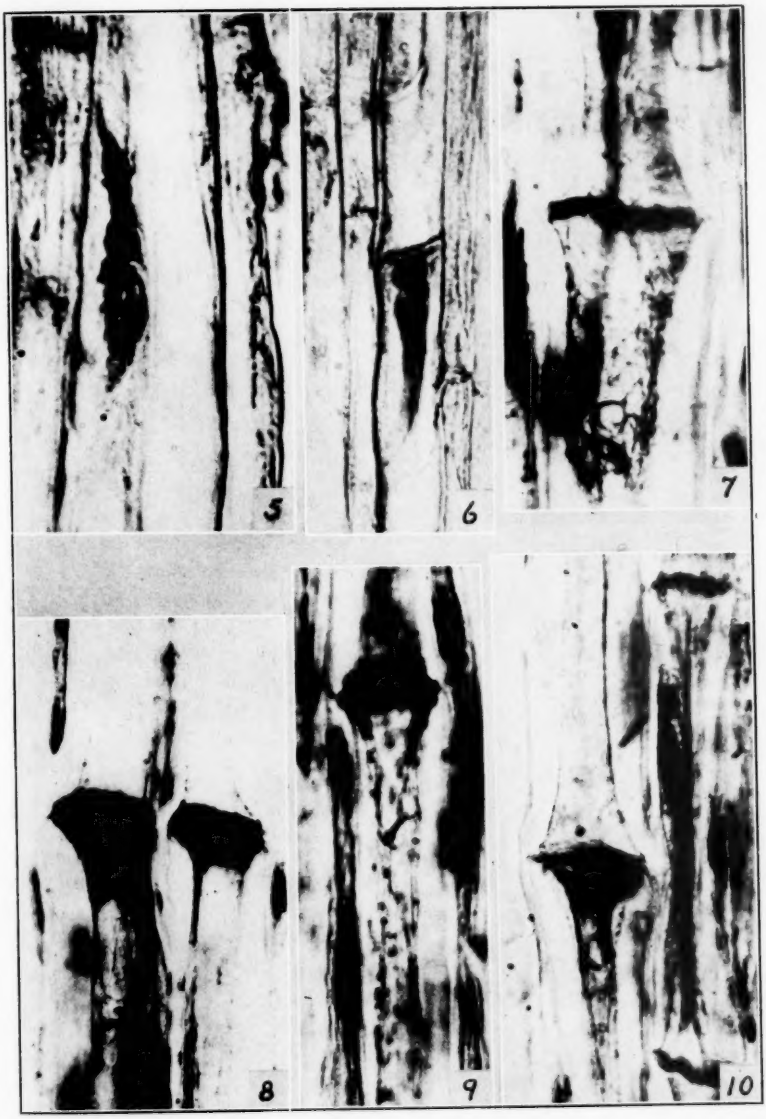
FIG. 6. Disintegrating slime body lodged against sieve plate. Figs. 5 and 6 stained with haematoxylin and light green. ×750.

FIG. 7. Disintegrating slime body, showing tangled mass of gelatinous strands lying within the cell lumen. ×900.

FIG. 8. Slime body further disintegrated. Strands have become threadlike, and much of the albuminous suspension has accumulated at the sieve plate. ×750.

FIG. 9. A few remnants of the slime body may be seen in this well developed sieve tube. Plastids have accumulated considerable carbohydrate material. ×750.

FIG. 10. Remnants of the slime body adhering to the internal protoplasmic structure of fully developed sieve tube. Figs. 7–10 are fresh hand-sections with IKI and anilin blue. ×750.



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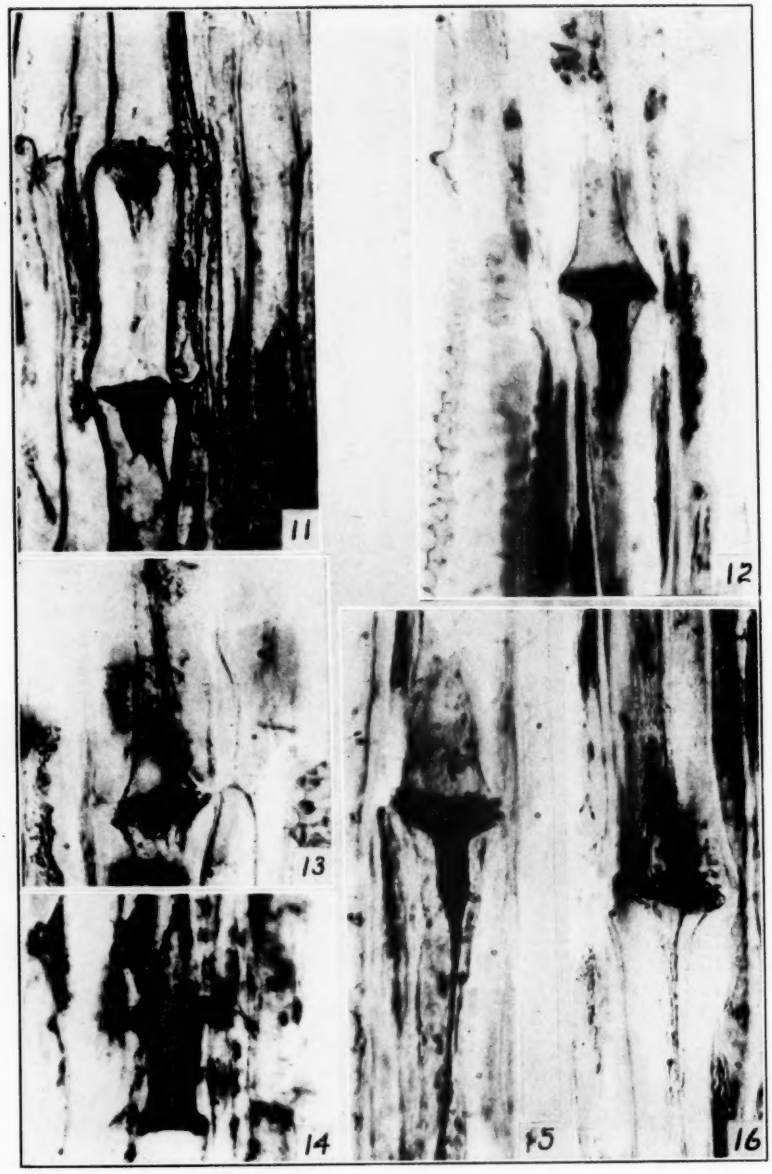

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PLATE IV

FIG. 11. Slime accumulations in young sieve-tube elements of potato stolon. Fragments of slime bodies may be seen within the accumulated masses. Haematoxylin and light green.

FIG. 12. Slime plug in a young, fully differentiated sieve tube. Parietal layer may be seen distinct from the slime.

FIG. 13. Slime accumulated as a thin, compact mass of homogeneous material lodged closely against the sieve plate. Internal protoplasmic strands may be seen projecting through the slime.

FIG. 14. Slime accumulation in a sieve tube killed by applying a mixture of 3 parts of absolute alcohol and 1 part glacial acetic acid to one end of the section. The mass is dense near sieve plate but less and less concentrated toward center of cell. Parietal layer is slightly contracted.

FIG. 15. Dense slime plug in old sieve tube formed by killing with 95 per cent. alcohol. Parietal protoplasm is contracted and surrounds the slime.

FIG. 16. Internal protoplasmic strands in mature sieve tube. Plastids above the plate. Figs. 12–16 stained with anilin blue and iodine. All $\times 750$.

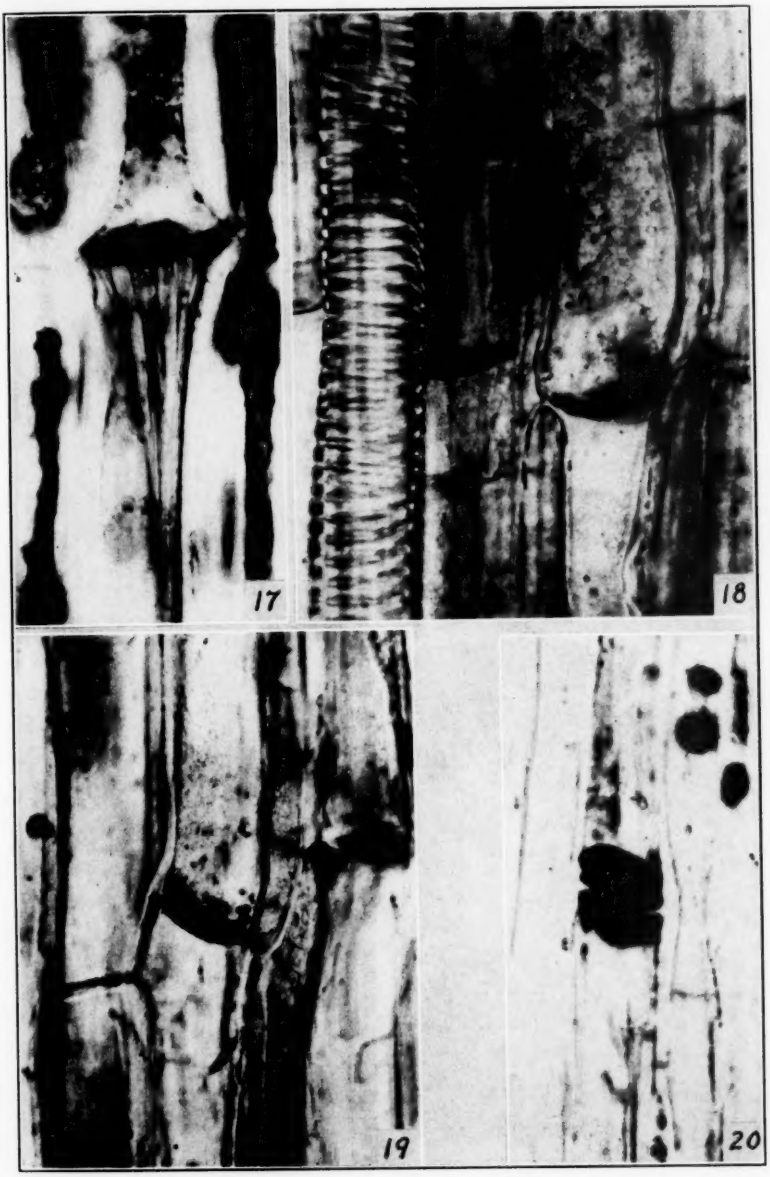
PLATE V

FIG. 17. Internal protoplasmic strands in mature sieve tube. Under the microscope the threads could be followed directly to the strands of sieve plate. Hand section killed and mordanted in IKI and stained with anilin blue. × 900.

FIG. 18. Sieve tube and spiral vessel of potato stolon. Section killed with absolute alcohol and acetic acid. Parietal and internal protoplasm unstained and not contracted. Sieve-tube lumen contents coagulated to a flocculent reticulum which completely fills the cell. Dark mass at the sieve plate is composed of plastids which were out of focus. × 750.

FIG. 19. Another sieve tube in same section as fig. 18, showing plastids more clearly. In living material these plastids are rapidly agitated by Brownian movement. Figs. 18 and 19 stained with anilin blue and iodine. × 750.

FIG. 20. Definitive callus on sieve plate stained with anilin blue. × 750.



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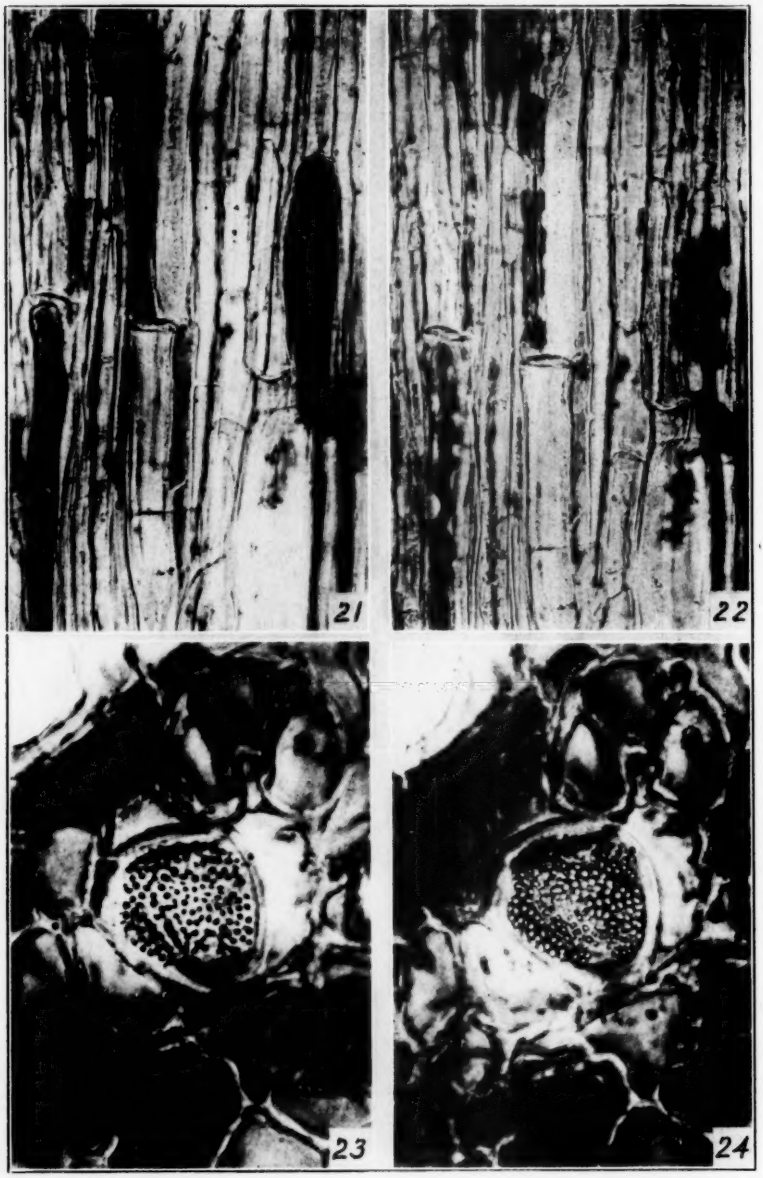

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PLATE VI

FIG. 21. Longitudinal section of potato stolon, showing sieve tube, companion cells, and phloem parenchyma in living condition after having accumulated neutral red for a short period of time. ×325.

FIG. 22. Same section after application of a hypertonic sucrose solution under the cover slip. Sieve tube in center of picture remains unchanged, while companion cells and phloem parenchyma are severely plasmolyzed. ×325.

FIGS. 23, 24. Sieve plate of potato stolon stained with iodine and anilin blue as viewed through a water immersion lens. With the focus high (fig. 23), the protoplasmic strands and surrounding callus cylinders appear as black spots. If the focus is slightly lower, the dark spots give way to small bright spots (fig. 24) which are considerably brighter than the unoccupied field of the microscope. ×900.

PLATE VII

FIG. 25. Young sieve plate, showing crater-like ends of callus cylinders which surround the protoplasmic strands. Stained with iodine and dilute anilin blue. ×800.

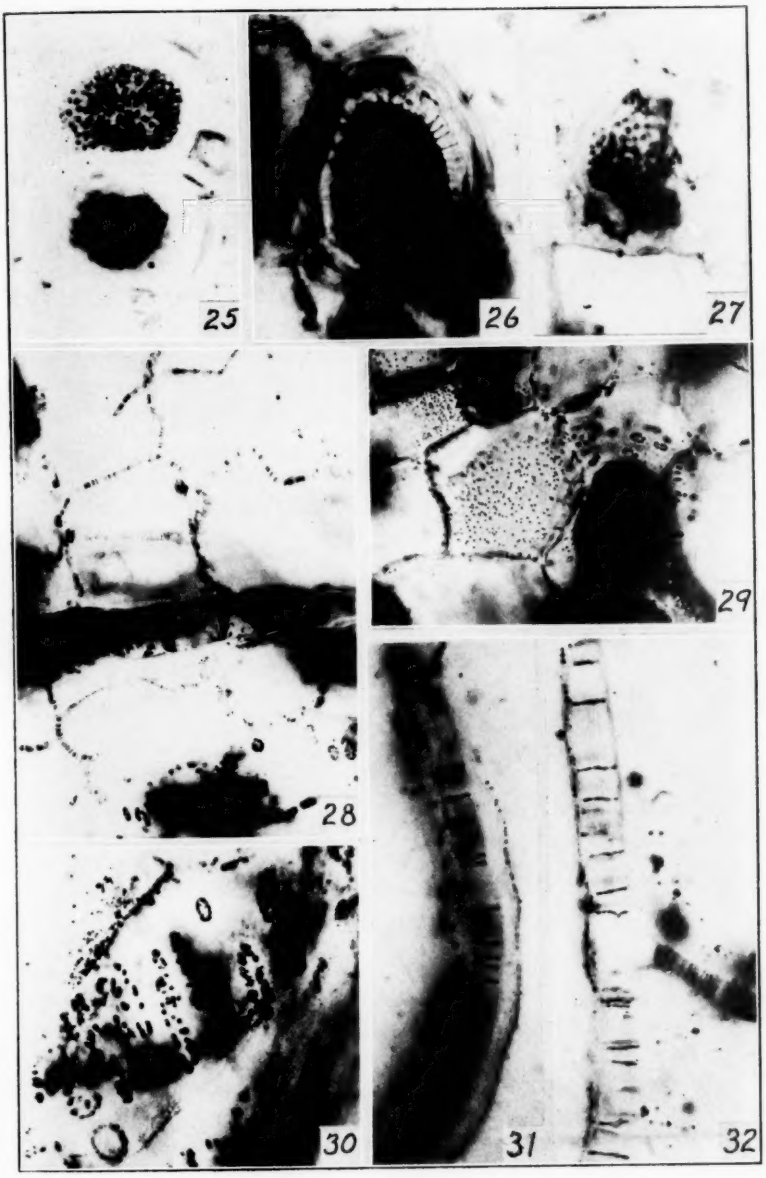
FIG. 26. Cup-shaped sieve plate, showing the narrow protoplasmic strands. Callus cylinders cannot be seen in this photograph because of lack of color differentiation; in the original section they could be seen. ×850.

FIG. 27. Transverse section showing sieve plate. Protoplasmic strands surrounded by callus cylinders. The sections in figs. 26 and 27 were stained with a strong solution of anilin blue after being mordanted in a very dilute iodine solution. Protoplasmic strands show black, and callus cylinders blue. A yellow filter was used in taking this photograph. ×850.

FIGS. 28, 29. Plasmodesma in potato tuber tissue. ×325.

FIG. 30. Plasmodesma of pits, more highly magnified. ×950.

FIGS. 31, 32. Thick walls of pith parenchyma of potato, showing plasmodesma in longitudinal view. Figs. 28–32 mordanted in iodine and stained with gentian violet in 5 per cent. sulphuric acid.



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PLASTID STUDIES IN GENETIC TYPES OF MAIZE: ARGENTIA CHLOROPHYLL

WILLIAM H. EYSTER[1]

(WITH TWELVE FIGURES)

The argentia chlorophyll pattern in maize has played an important part in the genetic studies of the writer since its discovery in 1918. Although a description of this chlorophyll character has already been published (**1**), it seems desirable, in view of the more recent but erroneous description of ZIRKLE (**4**), to point out again its principal characteristics. Argentia is a chlorophyll pattern which gives to the leaves a silvery appearance.

Argentia seedlings vary from pure albino to almost normal green in the stage when the first leaf emerges from the coleoptile, as may be seen from the illustration in figure 1. Chlorophyll development begins at the

FIG. 1. Maize seedlings showing argentia chlorophyll.

tips and bases of the leaves and extends longitudinally through the leaf, proceeding most rapidly along the vascular bundles. Consequently, the more or less albinotic leaf becomes transformed into an argentia leaf with green veins and colorless interveinal spaces.

Frequently in the late seedling or early plant stage, the argentia pattern disappears more or less completely, so that argentia plants cannot easily

[1] Fellow of the John Simon Guggenheim Memorial Foundation 1927–1928. Contributions from the Botanical Laboratory of Bucknell University, paper no. 5. Read before the American Society of Plant Physiologists at Cleveland, Ohio, December, 1930.

be distinguished from their normal green sibs. The pattern reappears as the plant approaches maturity, and may vary from a slight trace which is discernible only on the ventral side of the leaf tip to a full expression which extends throughout the entire leaf. A segment of a mature argentia leaf in comparison with a similar segment of a normal green leaf is illustrated in the description previously published (*loc. cit.*).

The nature of the argentia chlorophyll pattern in terms of chloroplastids may be seen from the leaf cross-section shown in figure 2. The chloro-

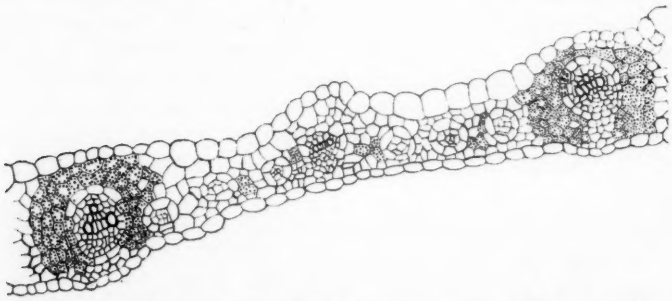

FIG. 2. Cross-section of a part of a leaf of an argentia plant with chlorophyll-bearing cells limited largely to the cells adjacent to the vascular bundles.

plastids in the cells adjacent to the conducting cells of the primary veins are of normal size (7 μ to 9 μ in longest diameter) and apparently contain a full complement of the chloroplastid pigments. Occasional cells adjacent to the conducting cells of the secondary veins also have plastids which appear to be normal in size and pigment content. The great majority of the cells between the principal veins, however, contain plastids which are less than 3 μ in diameter, and contain only traces of the chloroplastid pigments.

Argentia chlorophyll pattern as affected by environmental factors

The argentia pattern is highly variable in its expression under different environmental factors, and is especially sensitive to changes in temperature.

The relation between the development of the chloroplastids in argentia and temperature was clearly demonstrated for the first time in a greenhouse planting which extended across a steam trench. The soil directly over the steam trench was quite warm and the homozygous argentia seedlings growing in it were normal green in appearance, while individuals of the same progeny and of the same genetic constitution growing on either side of the trench had the argentia pattern clearly expressed. To check this observation, argentia seedlings of the same genetic constitution were grown under temperatures ranging from 10 to 40° C. Under the lowest temperatures,

the seedlings varied from white to cream in color as a result of the almost complete inhibition of chlorophyll development; in temperatures from 15 to 22° C., the argentia pattern was clearly expressed; while in temperatures over 25° C., the seedlings varied from some which had the pattern indistinctly expressed to others which were normal green in appearance. To facilitate the identification of the argentia plants in the genetic studies of maize in which this character is involved, the practice is to keep the greenhouse warm until the seedlings begin to appear through the soil and then to reduce the temperature to that of a semi-cold house.

Under average field conditions, the argentia pattern generally is distinct on the first three or four leaves. The following leaves are usually normal green and the pattern gradually disappears from the first leaves owing to the ultimate development of the chloroplastid pigments in the cells of the interveinal regions, so that the argentia plants can be recognized with difficulty or not at all in the late seedling or early plant stage. Unless the temperature continues abnormally high, the pattern is again expressed more or less clearly in the leaves between the earshoot and the tassel. The pattern is often distinct at the tip when it cannot be recognized in the other parts of the leaf.

A study was made also of the possible effect of light upon the expression of the argentia pattern. Light was found to affect argentia plants in the same manner as it affects green plants. When sufficient light for chlorophyll development is present, the seedlings vary in pattern according to the temperature in which they are growing. When a given temperature is maintained, the pattern cannot be changed markedly by varying the intensity of the light.

Plastid development and the argentia pattern

Under average or somewhat lower than average temperatures, the first leaves of argentia plants are practically colorless when they are first exserted from the coleoptile. Soon, however, the development of the chloroplastid pigments sets in, beginning at the tip and base of the leaf, and gradually the leaf assumes the argentia pattern, and may finally become uniformly green. In order to determine the nature of these changes, cross-sections of leaves in various stages of development were studied. In the young, colorless leaves, the plastids are small and lack pigments. Chloroplastid development proceeds more rapidly in the cells adjacent to the conductive cells of the vascular bundles than in the other cells of the leaf, and most rapidly in the cells adjacent to the conductive cells of the midrib.

Visible plastids are first apparent in the cells adjacent to the vascular cells which make up the midrib of the leaf, as shown in figure 3. The plastid pigments are first discernible when the plastids are from 2 μ to 3 μ

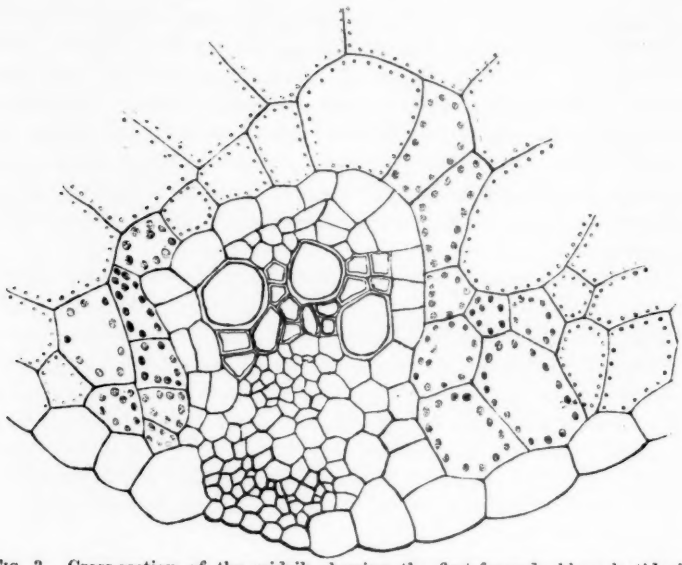

FIG. 3. Cross-section of the midrib showing the first-formed chloroplastids in the leaf.

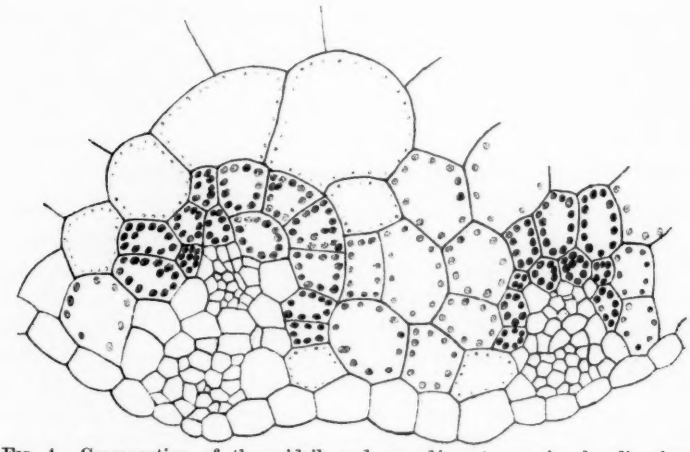

FIG. 4. Cross-section of the midrib and an adjacent vascular bundle showing a later stage in the development of the chloroplastids.

in longest dimension. Plastids become apparent next in the cells adjacent to the conductive cells of the principal vascular bundles on each side of the midrib. The plastids of the cells which ensheath the vascular bundles

have developed sufficient amounts of the chloroplastid pigments to appear green while the plastids in the cells between the bundles are still small and colorless, thus giving to the leaf the argentia pattern, which consists in the longitudinal extension of green veins through an otherwise white or cream-colored leaf blade. A somewhat later stage in the development of the chloroplastids in the region of the midrib of the leaf is shown in figure 4. The plastids in the principal and secondary veins are approaching mature size and have enough chlorophyll to appear green while the plastids in the cells between the veins are small and the pigments are just beginning to appear.

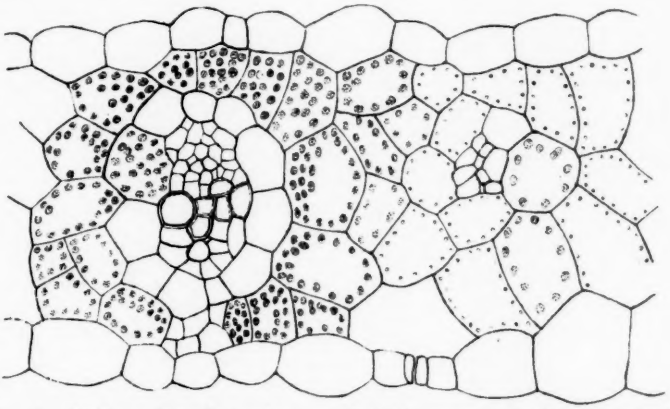

FIG. 5. Section of leaf showing a principal vascular bundle surrounded with chlorophyll-bearing cells while the plastids are just beginning to develop pigments in the cells around the secondary vascular bundle.

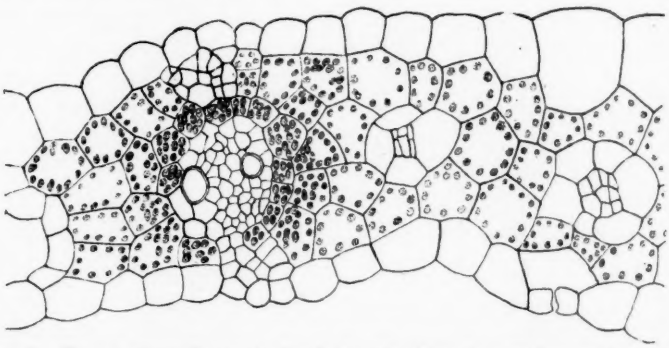

FIG. 6. Cross-section of a part of a leaf with pigments well developed in the cells around the vascular bundles, while the cells in the interveinal regions are beginning to develop the plastid pigments.

Plastid development proceeds more rapidly at the tip than at the base of the leaf. A cross-section of a leaf tip, which is beginning to show the argentia pattern, is illustrated in figure 5. The plastids of the cells adjacent to the conductive cells of the vascular bundles hardly reach mature size and full pigment content before the plastids of the cells in the interveinal regions become pigmented as is indicated in the drawing in figure 6. Soon the plastids of the cells of the interveinal regions attain normal size and pigment content, and the leaf tip becomes uniformly green. A cross-section of a green leaf tip of an argentia leaf is shown in figure 7. This progressive development of the plastids continues until the entire leaf becomes green.

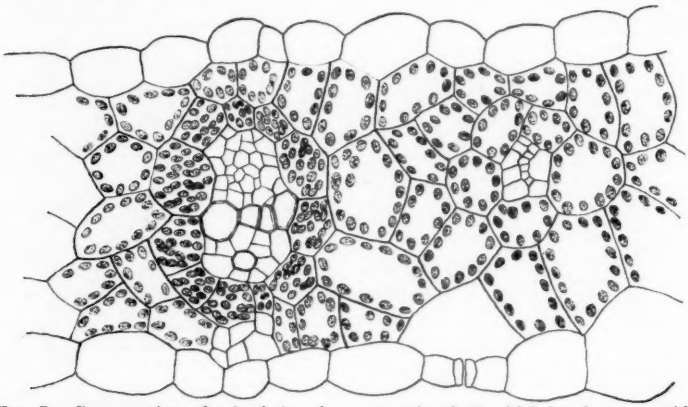

FIG. 7. Cross-section of a leaf tip of an argentia plant which has become uniformly green.

Inheritance of the argentia chlorophyll pattern

The argentia chlorophyll pattern is inherited as a simple Mendelian recessive character, and is the expression of a gene in the C-Sh-Wx chromosome as shown in a previous publication (*loc. cit.*). Back-cross progenies, including 12,986 individuals, showed a linkage between shrunken endosperm and the argentia chlorophyll pattern with 26.03 per cent. crossing over.

More recently, linkage tests have been made between argentia chlorophyll and waxy endosperm, with the results shown in table I. F_1 plants of the constitution $\frac{Wx\ ar}{wx\ Ar}$ were back-crossed to double recessives. The progenies from these back-crosses included 2631 plants, and, of these, 286 had the characters in question in new combinations as a result of crossing over. These results indicate a linkage between waxy endosperm and argentia with 10.87 per cent. crossing over.

F_1 progenies from the back-cross $\frac{Wx\ Ar}{wx\ ar} \times \frac{wx\ ar}{wx\ ar}$ are listed in table II.

TABLE I

F_1 PROGENIES FROM THE BACK-CROSS $\frac{Wx\ ar}{wx\ Ar} \times \frac{wx\ ar}{wx\ ar}$

PEDIGREE	*Wx Ar*	*Wx ar*	*wx Ar*	*wx ar*	TOTAL
9379–65	21	166	142	19	348
–68	28	128	210	33	467
–74	20	144	210	11	385
–75	36	242	255	25	558
–79	38	218	235	20	511
–86	25	166	161	10	362
Total	168	1132	1213	118	2631

These progenies, which involve *wx* and *ar* in the coupling phase, show a linkage between argentia and waxy endosperm with 10.75 per cent. crossing over. Of the 4075 plants observed, 438 plants had these characters in new combinations as a result of crossing over.

The locus of *wx*, according to EYSTER (**1**), is at 45.06. The average cross-over value between *wx* and *ar*, as determined from the data in tables I and II, is 10.81. This value may be taken as the map distance between *wx* and *ar* in the chromosome. Since the cross-over value between *sh* and *ar* is greater than that between *sh* and *wx*, it follows that the locus of *ar* is on the opposite side of *wx* from *sh*. Accordingly, the locus of *ar*, as measured from *wx*, is at 55.78. (See fig. 8.)

TABLE II

F_1 PROGENIES FROM THE BACK-CROSS $\frac{Wx\ Ar}{wx\ ar} \times \frac{wx\ ar}{wx\ ar}$

PEDIGREE	*Wx Ar*	*Wx ar*	*wx Ar*	*wx ar*	TOTAL
10754–79 × 10769–55	249	34	37	257	577
" –23 × " – 2	297	32	35	343	707
" –39 × " –13	313	32	30	271	646
" –54 × " –25	280	44	43	260	627
" –45 × " – 4	288	28	25	252	593
" –81 × " –52	153	17	21	136	327
" –20 × " – 2	285	31	29	253	598
Total	1865	218	220	1772	4075

Chloroplastid pigments in the argentia plants

Analyses of the chloroplastid pigments in argentia leaves were made after the methods of WILLSTÄTTER and STOLL (**2, 3**) with modifications and adaptations as described elsewhere by the writer (in manuscript).

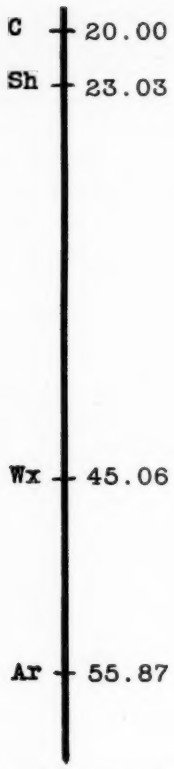

FIG. 8. Chromosome map of a section of the C–Sh–Wx chromosome to show the locus of the Ar gene.

TABLE III

TOTAL CHLOROPHYLL IN GREEN AND ARGENTIA PLANTS

EXPERIMENT	CHLOROPHYLL TYPE	FRESH WEIGHT	TOTAL CHLOROPHYLL	
			TOTAL	PER GRAM
		gm.	*mg.*	*mg.*
7a	Green	6.430	9.0695	1.410
8a	Green	7.645	9.1630	1.200
9b	Argentia	12.570	9.2395	0.735
10b	Argentia	10.300	7.0125	0.681
11b	Argentia	6.100	7.0452	1.160
12b	Argentia	13.230	13.8386	1.046

Determinations of total chlorophyll were made from extracts of fresh leaf tissue of (1) argentia seedlings, and (2) green plants of the same pedigrees. The results of these determinations are given in table III. The green plants contained from 1.20 to 1.41 mg. of chlorophyll per gram of leaf tissue, while the argentia plants contained from 0.681 to 1.16 mg. per gram of leaf tissues. This variation in the total chlorophyll content was due apparently to the differences in temperature under which the seedlings were grown and to differences in the age of seedlings when analyzed. The chlorophyll content of the green plants was, on the average, 1.441 times as much as that of the argentia plants. The rather wide variation in chlorophyll content of the argentia plants was to be expected because of the variability in the expression of the argentia pattern.

Analyses were also made of dry leaf powder of (1) argentia plants and (2) green plants of the same genetic strain. (See table IV.) The total

TABLE IV

TOTAL CHLOROPHYLL IN GREEN *vs.* ARGENTIA PLANTS

EXPERIMENT	CHLOROPHYLL TYPE	PEDIGREE	DRY WEIGHT	TOTAL CHLOROPHYLL	
				TOTAL	PER GRAM
			gm.	*mg.*	*mg.*
1a	Green	10295	2.331	13.1791	5.6538
1b	Argentia	"	2.214	10.4090	4.7014
2a	Green	"	1.982	9.1133	4.5980
3a	Green	10299	1.548	10.4877	6.7750
3b	Argentia	"	1.976	7.6157	3.8541
4a	Green	"	1.548	10.9512	7.0744
5a	Green	"	2.191	8.1639	3.7261
5b	Argentia	"	1.822	8.7473	4.8009

chlorophyll content of the green plants varied from 3.7261 to 7.0744 mg. per gram of dry leaf powder, while that of the argentia plants varied from 3.8541 to 4.8009 mg. per gram of leaf powder. A summary comparison of these results indicates that the green plants had, on the average, 1.392 times as much chlorophyll as the argentia plants.

Studies were also made of the individual plastid pigments of green and argentia plants belonging to the same genetic strain. The individual pigments occur in the green as well as in the argentia plants in the general proportions as established by WILLSTÄTTER and his coworkers, as may be seen from the results tabulated in table V. Since the chloroplastids of the argentia plants apparently have the pigments in the same proportions as the green plants, it follows that the gene *ar* inhibits the development of all pigments alike. The relation of chlorophyll *a* to chlorophyll *b*, which may

TABLE V

INDIVIDUAL PLASTID PIGMENTS IN GREEN AND ARGENTIA PLANTS, WITH AND WITHOUT ENDOSPERM

EXP.	ENDOSPERM	DRY WEIGHT PER 100 GRAMS FRESH WEIGHT	PIGMENT PER GRAM DRY LEAF TISSUE			
			CHLOROPHYLL		CAROTIN	XANTHOPHYLL
			a	b		
		gm.	*mg.*	*mg.*	*mg.*	*mg.*
Ar	Present	8.14	10.8120	2.5671	0.07975	0.12083
ar	"	8.16	8.4891	2.6626	0.14898	0.22199
Ar	Removed	8.20	7.7636	2.4158	0.16740	0.26214
ar	"	8.22	8.2689	2.9579	0.14740	0.22677

be expressed as Q a/b, was found to be 4.21 in the green plants and 3.19 in the argentia plants. This ratio was greatly reduced in plants from which the endosperm had been removed as soon as the first leaf was exserted from the coleoptile. In green plants treated in this manner, the Q a/b was 3.214 while that of argentia plants similarly treated was 2.80. These results would seem to indicate that removing the carbohydrates which are normally available in the endosperm produced a lower value of Q a/b by decreasing the rate of development of chlorophyll *a*.

The relation of carotin to xanthophyll, expressed by Q c/x, was found to be 0.66 in the green plants and 0.67 in the argentia plants. In plants from which the endosperm had been removed when the first leaf was exserted from the coleoptile, the value of Q c/x for green plants was 0.64 and for argentia plants was 0.65. From these comparisons it is concluded that the removal of the endosperm had no effect upon the rate of development of carotin and xanthophyll.

Chloroplastid pigments and carbon assimilation

A preliminary study of the photosynthetic efficiency of the argentia plants in relation to that of the green plants of the same genetic strain was made by comparing their dry weights. The entire plants were dried in a vacuum desiccator and weighed on a sensitive balance.

The dry weight of young seedlings, based upon a large number of individuals, was determined to be 8.14 per cent. of the fresh weight in green plants, and 8.16 per cent. of the fresh weight in argentia plants. When plants are in the four-leaf stage, the mean dry weight is 6.97 per cent. of the fresh weight in green plants and 6.10 per cent. of the fresh weight in argentia plants. The mean dry weight in pedigree 10335 was 93.5 mg. for green plants and 80.3 mg. for argentia plants.

Some of the plants of pedigree 10337 were dried and weighed at the stage in development when the food reserve in the endosperm had just been exhausted. The mean dry weight of the green plants was 82.253 mg. and that of the argentia plants was 76.840 mg. The remainder of the plants were grown until the fourth leaf was full developed, when they were cut off at the level of the ground, dried, and weighed. The mean weight of these older plants was 140 mg. for the green and 74.75 mg. for the argentia plants.

A summary comparison of these dry weights shows the green plants to be 1.3684 times as heavy as the argentia plants. This weight relationship between green and argentia plants is in close agreement with the relation in total chlorophyll content, which was found to be 1.441 as much in green plants as in argentia plants in extracts from fresh leaf tissue and 1.392 times as much in green as in argentia plants in extracts from dried leaf powder.

Removal of endosperm and dry weights in green and argentia seedlings

In order to study more closely the relation between chlorophyll content of green and argentia seedlings and increases in dry weight, the endosperm was removed from the seedlings of alternate rows in the seed bed at the stage in development when the first leaf was newly exserted from the coleoptile. The seedlings were then kept under conditions favorable for growth until the endosperm in the seedlings from which it had not been removed had been completely digested and absorbed by the growing plants. The young plants were then cut off at the level of the ground, dried in a vacuum desiccator, and weighed individually on a sensitive balance. The results of these weighings are given in table VI, and are compared graphically in figures 9 and 12.

The mean weight of 162 green seedlings was 68.40 ± 1.63 mg. while the mean weight of 209 argentia seedlings of the same genetic strain was 59.96 ± 1.08 mg. This difference of 12.44 ± 1.96 mg. presumably was due to the higher chlorophyll content of the green plants. The weight coefficient of *Ar/ar* is 1.22, and is in close agreement with the total chlorophyll coefficient of *Ar/ar*, which was found to be 1.20. This comparison is shown by the curves in figure 9.

The mean weight of 190 green seedlings from which the endosperm had been removed was 61.05 ± 1.65 mg., while the mean weight of 162 green seedlings from which the endosperm had not been removed was 68.40 ± 1.63. This is a difference of 7.35 ± 2.314 mg., and is doubtless due to the greater chlorophyll content of the seedlings from which the endosperm had not been removed. This comparison is indicated graphically in figure 10.

TABLE VI

EFFECT OF THE REMOVAL OF ENDOSPERM UPON DRY WEIGHT OF GREEN AND ARGENTIA MAIZE PLANTS

EXPERIMENT NUMBER	GENETIC TYPE	ENDOSPERM REMOVED	CLASS CENTERS OF DRY WEIGHT PER PLANT IN MILLIGRAMS															TOTAL
			5	15	25	35	45	55	65	75	85	95	105	115	125	135	145	
13a	*Ar*	No	1	6	13	14	12	17	24	21	19	5	16	2	7	4	1	162
13b	*ar*	No	1	9	23	28	37	27	25	23	12	12	8	1	2	1	...	209
14a	*Ar*	No	1	6	13	14	12	17	24	21	19	5	16	2	7	4	1	162
14b	*Ar*	Yes	1	19	13	30	24	17	13	23	10	13	8	6	5	5	3	190
15a	*ar*	No	1	9	23	28	37	27	25	23	12	12	8	1	2	1	...	209
15b	*ar*	Yes	0	5	24	27	38	30	13	15	13	11	4	2	2	1	1	186
16a	*Ar*	Yes	1	19	13	30	24	17	13	23	10	13	8	6	5	5	3	190
16b	*ar*	Yes	0	5	24	27	38	30	13	15	13	11	4	8	2	1	1	186

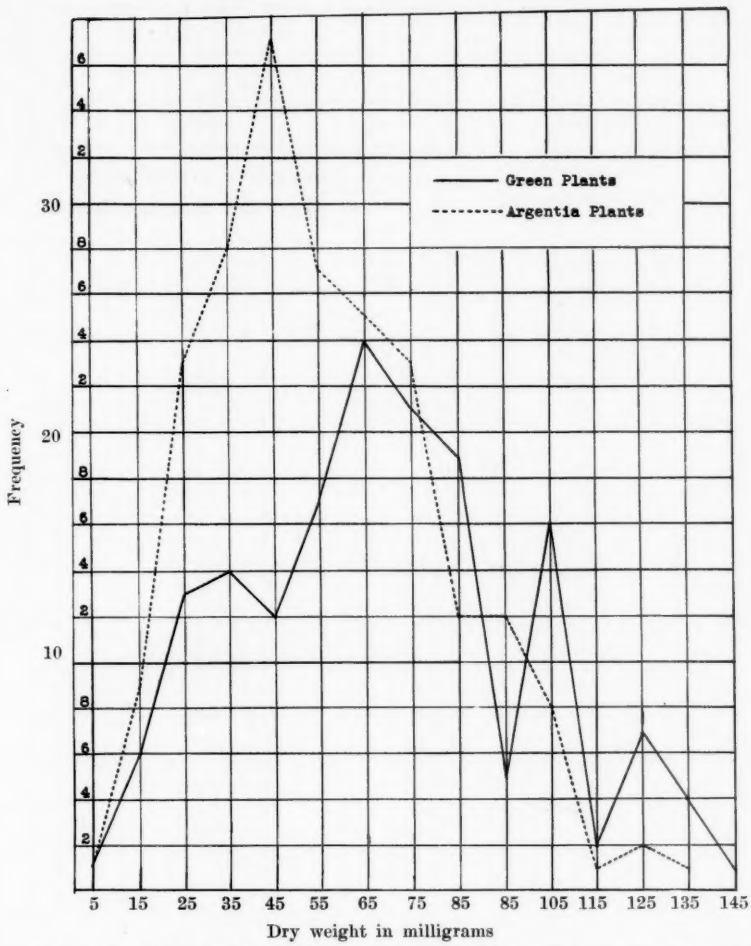

FIG. 9. Variation in dry weight of green and argentia seedlings of the same genetic strain.

A comparison was also made between green and argentia plants from which the endosperm had been removed as already described. The mean weight of 190 green plants was 61.05 ± 1.65 mg., while that of 186 argentia plants was 55.27 ± 1.27 mg. This difference in the mean weight of green and argentia plants is less than three times the probable error of the difference. Since the argentia plants have fewer pigmented plastids than the green plants, these results would seem to show that the chloroplastids of

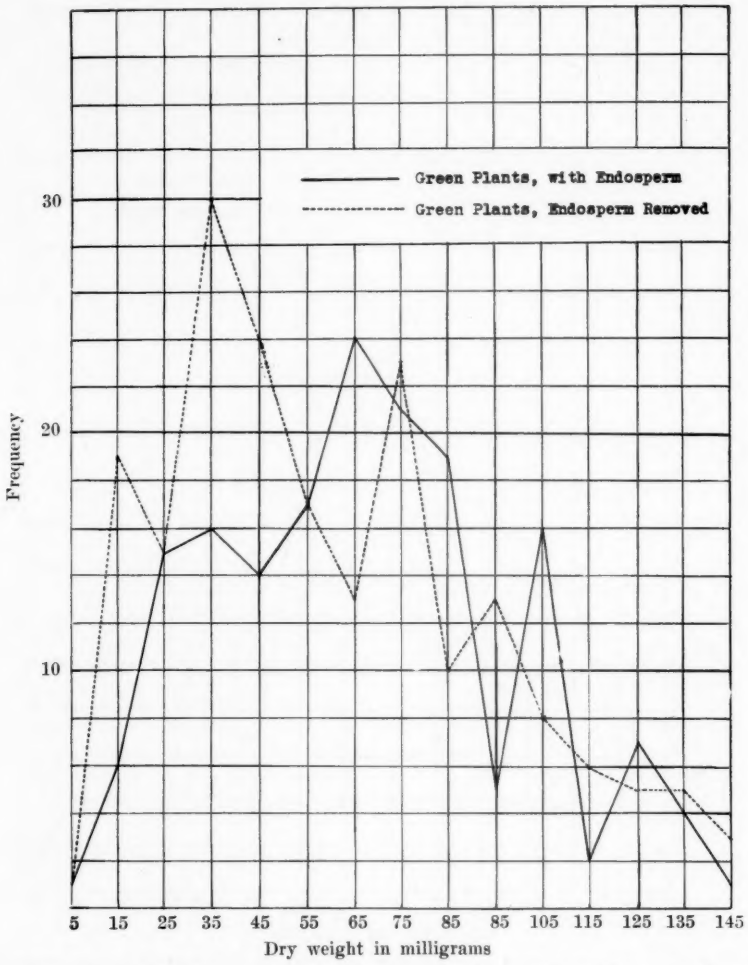

FIG. 10. Comparison in dry weight of green plants with endosperm and green plants from which endosperm had been removed in early seedling stage.

the argentia plants have a greater photosynthetic efficiency than those of the green plants from which the endosperm had been removed in the early seedling stage. Although chlorophyll determinations were not made for seedlings from which the endosperm had been removed, it is probable that chlorophyll development is retarded somewhat in the green plants thus treated. A graphic representation of the dry weights of green and argentia

plants respectively from which the endosperm had been removed is given in figure 11.

The argentia plants from which the endosperm had been removed increased in dry weight as rapidly as those which possessed endosperm, as shown graphically in figure 12. The mean weight of 209 plants which had an endosperm was 55.96 ±1.08 mg., while the mean weight of 186 argentina

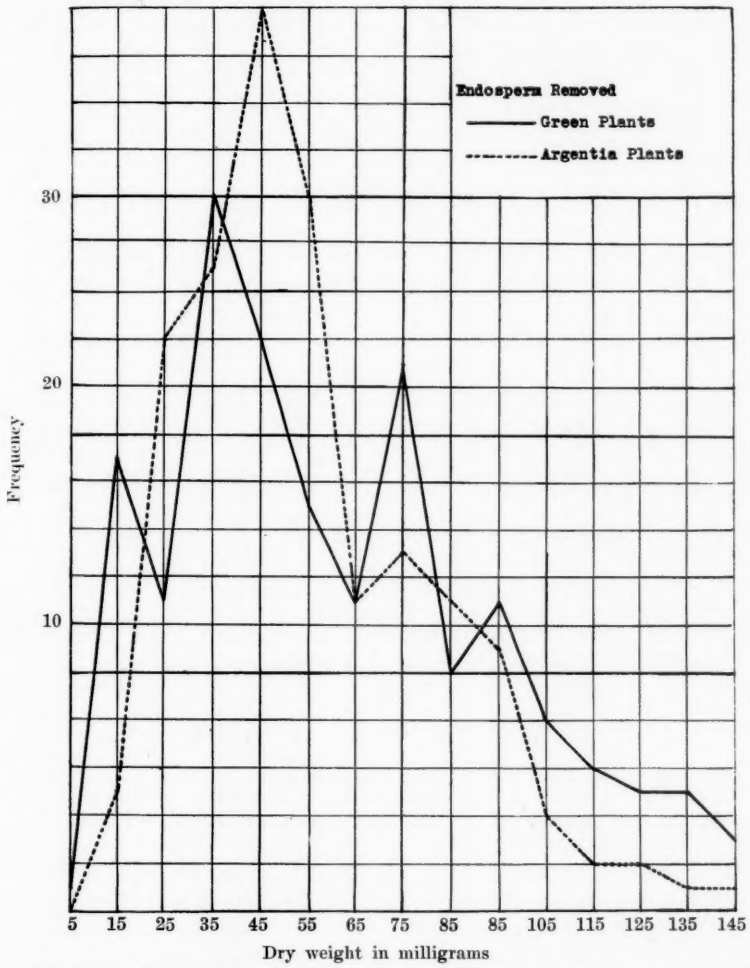

FIG. 11. Comparison in dry weight of green and argentia plants from which endosperm had been removed.

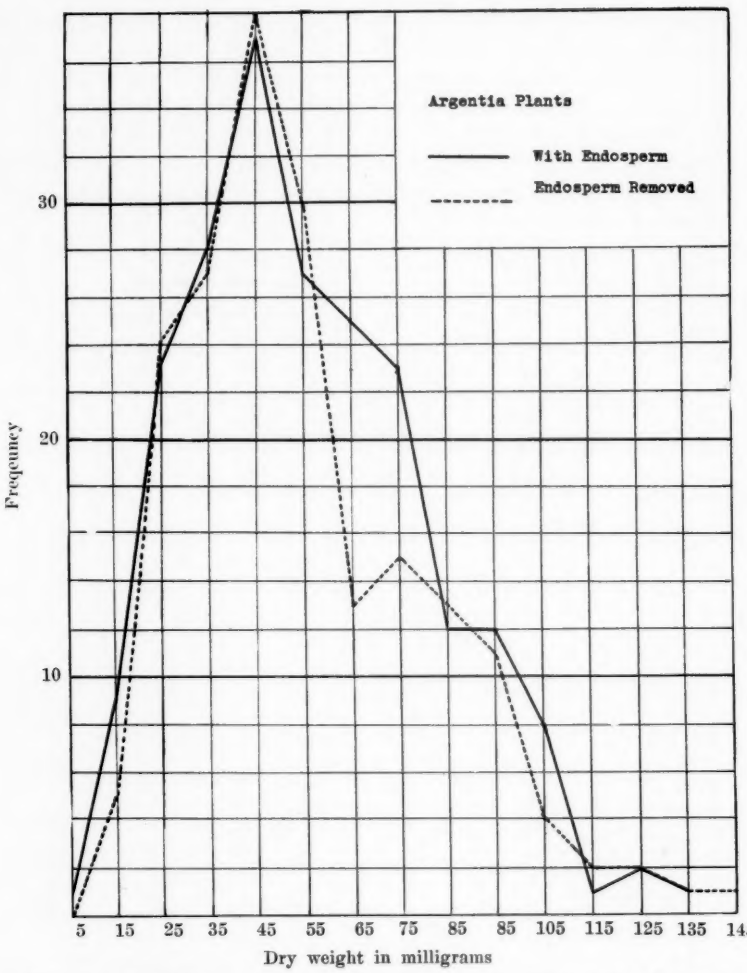

FIG. 12. Comparison in dry weight of argentia seedlings with endosperm and argentia plants from which the endosperm had been removed in early seedling stage.

plants from which the endosperm had been removed was 55.27 ± 1.27 mg. This is a difference of only 0.69 ± 1.67 mg. This result was to be expected, since the argentia plants which lacked endosperm contained as much chlorophyll as those which possessed an endosperm. Why chlorophyll development is not influenced by the removal of the endosperm in the argentia plants,

but is greatly reduced in the green plants when the endosperm is removed in the early stage has not been determined.

Summary

1. The expression of the argentia chlorophyll pattern is influenced by temperature.

2. The argentia chlorophyll pattern is the result of the more rapid development of the chloroplastids in the cells which are adjacent to the vascular bundles. The plastids in the other cells of the leaf mesophyll develop pigments tardily or not at all.

3. The argentia chlorophyll pattern is inherited as a simple Mendelian recessive character. It is the expression of a gene which has its locus in the C-Sh-Wx chromosome at 10.81 cross-over units to the right of the locus of Wx. The order of the genes in this chromosome is C-Sh-Wx-Ar, as illustrated in figure 8.

4. Argentia seedlings have less total chlorophyll than green plants of the same genetic constitution.

5. The chloroplastid pigments occur in argentia plants in the same proportions as in the green plants.

6. Chlorophyll content is greatly reduced in green plants by the removal of the endosperm at the stage in development when the first leaf is exserted from the coleoptile.

7. Chlorophyll content in the argentia plants is not affected by the removal of the endosperm.

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EFFECT OF SEVERAL ENVIRONMENTAL FACTORS ON THE HARDENING OF PLANTS[1]

S. T. DEXTER[2]

(WITH FIVE FIGURES)

In several papers (**1–3**), DEXTER, TOTTINGHAM, and GRABER have outlined a method for determining the injury which plants suffer as a result of freezing. This method involves the determination of the exosmosis of mineral matter from the injured tissues, by measurement of the electrical conductivity of the water in which they have been immersed. Since the method is very sensitive to changes in the hardiness of plants, it appeared that the influence of various environmental conditions on hardening could be readily studied. This paper deals with the influence on hardening of light of different day-lengths, and of darkness. The influence of light with and without carbon dioxide (*i.e.*, with and without photosynthesis) was also studied. Several constant temperatures were used, and various combinations of alternating temperatures, both with and without carbon dioxide. Alfalfa, wheat, cabbage, and tomato plants were used for experimental material.

Experiment 1

On August 25, pots of alfalfa plants of an especially uniform Turkestan strain were transferred from the greenhouse to a large cold room. These plants were grown from seed sown in May, and furnished by Professor L. F. GRABER, of the University of Wisconsin. The pots were one-quart ice cream cartons, paraffined thoroughly. There were generally five plants in each. The plants had never been defoliated. For this experiment, plants under four conditions during hardening were studied: (1) The tops were removed from the plants, and the pots were kept at 0° C. in darkness. (2) The tops were not removed, but the plants were kept in darkness at 0° C. (3) The tops were not removed, and the plants were illuminated for seven hours each day, in a gradually changing temperature which reached a maximum of about 10° C. after four hours of illumination; when the lights went off, the temperature gradually fell to 0° C. Two 1000-watt lamps were used, giving an intensity at the pots of from 900 to 1200 foot-candles, according to the Macbeth illuminometer. Thermograph records were kept. (4) The tops were not removed, but the plants were kept at 0° C. both during illumination and while in the dark. The illumination period was for seven hours, with a slightly greater intensity than in (3).

[1] Contribution from the Hull Botanical Laboratory.

[2] National Research Council fellow in the Biological Sciences.

The temperature at the pots always rose a degree or two when the lights went on, in spite of very thorough stirring of the air with an oscillating 12-inch electric fan.

Each sample tested consisted of the roots of the plants from two pots, trimmed to weigh 5 grams when clean and dried of surplus surface water. The plants were very uniform. The samples were placed in 1×8-inch pyrex test-tubes and frozen in an alcohol-ice slush bath at −7° C. for four hours. They were thawed by immersion of the tubes in a water-bath at 2° C. for one hour. After thawing, 25 cc. of distilled water at 2° C. were added to each tube in turn. Exosmosis was permitted to continue for 20 hours at 2° C., when the liquid was drawn off and the electrical conductivity determined at that temperature. Table I shows the results of these determinations after various periods of hardening treatment.

TABLE I

SPECIFIC CONDUCTIVITIES (×10^6, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF ALFALFA ROOTS FROZEN FOR FOUR HOURS AT −7° C. INTERVAL OF 20 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

TREATMENT AND CONDITION	DAYS OF HARDENING TREATMENT					
	0	4	7	14	28	42
1. Tops removed, dark, 0° C.	755	845	840	502	551	515
2. Tops not removed, dark, 0° C.	755	894	817	478	509	537
3. Tops not removed, 7 hours' light, alternating temperature	755	799	573	472	389	408
4. Tops not removed, 7 hours' light, constant temperature	755	662	577	468	346	341

Figure 1 shows these results in graphic form. It would appear that the four conditions divide roughly into two classes. The plants in the dark hardened much the same, whether with or without tops; those in the light hardened finally much the same, whether with constant or alternating temperature. At the end of 10 days, since the division into two groups was then becoming evident, two pots were transferred from condition no. 4 into a constant temperature (0° C.) in the dark, with tops removed. At the end of the experiment, these roots gave a conductivity reading of 551, as against 341 for those continuing in the light. This indicated clearly that hardening had not continued in the dark chamber, although it was well started in the light. Another pair of pots was transferred from condition no. 1 and from condition 2 into the dark but with the alternating tempera-

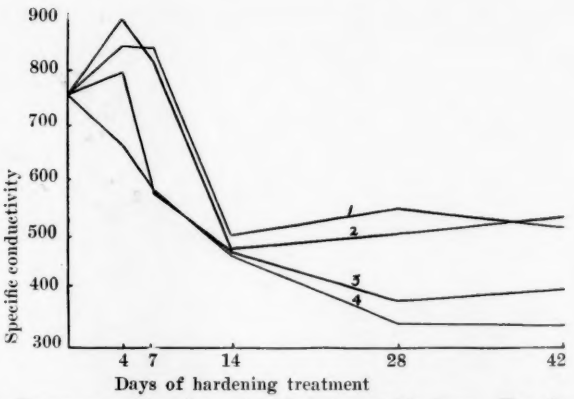

FIG. 1. Curves presenting data of table I in graphic form. The plants in treatment 4 (continuous cold, with light) hardened the most rapidly and the most completely; those in treatment 3 (alternating temperatures, with light) hardened almost as much. Plants in treatments 1 and 2 (in the dark) hardened much less than those in treatments 3 and 4.

ture of condition 3. They gave an average conductivity value of 517 at the end of 42 days. Thus hardening was not notably more efficient with the alternating temperatures in the dark than with the constant temperature in the dark.

On the last date, after 42 days of hardening treatment, the samples were so prepared that buds were left at the crown, in order that recovery in the greenhouse could be noted after freezing and exosmosis. Recovery of the plants showed that the treatments could be readily distinguished. Plants receiving light during hardening made much greater growth and fewer plants died.

REPETITIONS OF EXPERIMENT

Experiment 1 was twice repeated, with variations. In each case alfalfa plants in darkness hardened less completely than those in the light. Various day-lengths were used in the hardening chamber at 0° C. and at 10° C. The samples in these experiments were far from uniform, and definite conclusions could not be drawn. In general, hardening was favored by a long period of illumination, if the plants were kept continuously in the cold. In the case of alternating temperatures during hardening treatment, hardening was retarded by the long day at higher temperatures (PELTIER and TYSDAL 5), and elongation of stems was favored. Under this treatment, elongation of stems was especially evident if the plants had been given a short day (7 or 8 hours) previous to such hardening treatment. Figure 2 shows the change that occurred in previously short-day alfalfa plants during

FIG. 2. Condition of alfalfa plants, grown for several months in the greenhouse with a 7 or 8-hour day and then transferred to hardening rooms. The pot on the left received 14 hours of light each day at 0° C., and was kept in the cold continuously; the pot on the right received 14 hours of light each day at a room temperature of about 22° C. The first pot made no evident growth, while the second grew several inches in 21 days. The first treatment gives good hardening, while the second treatment stimulates top growth and almost prevents hardening.

a treatment in which they were given 14 hours of illumination at 22° C. for 21 days, with 10 hours of each day in the dark at 0° C. While such plants continuously in the cold room (0° C.), with 14 hours of illumination, hardened from a specific conductivity of 889 at the start to 559 at the end of 21 days, similar plants receiving equal light in the warm room hardened to only 751. The same general relation was found in the case of plants which received light for about 14 hours each day previous to hardening. These plants hardened from 797 to 497 when kept continuously in the cold room with 14 hours of light, while similar plants receiving 14 hours of light in a room at 22° C., and 10 hours in the dark at 0° C. each day, hardened to only 652. The short-day plants shown had received 7 or 8 hours of daylight in the greenhouse each day for several months previous to hardening treatment; the long-day plants had received 14 or 15 hours of illumination each day for the same period.

In no case did alfalfa plants harden as completely in the dark as in the light. Alternating temperatures were no more effective than continuous ones in any case studied. If the illumination tended to favor much extension of parts, hardening was retarded or prevented. Hardening was more

thorough at 0° C. than at 10° C. Although these plants were in general very well stored with starch and other reserve foods, as shown by microinspection, hardening was always more complete in the light, when photosynthesis could furnish a continually replenished supply of carbohydrates for the processes of the plants.

Experiment 2

In this experiment, Minhardi winter wheat was grown in perforated soil-temperature cans (6 × 9-inch cylinders) in soil which was thoroughly sifted and mixed. The seed was sown on November 25, the plants were thinned to 15 plants per can, and, from the first, were exposed to two day-lengths in the greenhouse, at about 62° F. One set received ordinary daylight (reinforced with Mazda lamps on especially dark days) for 8 hours each day; the other set received the full day-length, with lights (four 200-watt, about 2 feet away) extending the illumination period until 10 P. M., which gave approximately a 14-hour day. At an age of six weeks, the short-day plants were much smaller and somewhat greener than the long-day plants. Fifteen short-day plants weighed, entire, 3.78 grams; 15 long-day plants, 7.0 grams. The short-day plants (leaves) had a percentage dry matter of 14.0; the long day, 13.0. The percentage ash (wet-weight basis) was slightly higher in the short-day plants than in the long-day plants, 2.22 as against 1.96. The electrical conductivity of the ash solution, after converting the basic oxides to carbonates and made to volume on a wet-weight basis, was about 2 per cent. higher in the short-day plants.

These plants were taken to the hardening chamber on January 6 (six weeks old), where several pots from each set (long- and short-day) were put to harden in the following ways: (1) in the dark; (2) with 7 hours of light each day; (3) with 15 hours of light each day. All were at 0° C. Two 1000-watt lamps furnished the light, as in the preceding experiment. Precaution was taken to avoid injury to the plants by lowering the temperature of the room gradually, taking about 6 or 7 hours. Very little wilting was noted. The samples were prepared by removing the roots and leaf blades, and washing and drying the remaining crowns. Duplicate samples weighed 0.9 gram each; they were frozen for 2 hours at −10° C. in an alcohol-slush bath and thawed for 30 minutes in the water-bath at 2° C. Fifteen cc. of distilled water (2° C.) was then added to each tube. Exosmosis went on for 16 hours at 2° C., when conductivity measurements were made. Table II shows the conductivity values obtained in this experiment after the durations of hardening treatment indicated.

Table II shows consistent results throughout. The plants, whether long- or short-day in the greenhouse, hardened little if at all in the dark; they hardened materially in 7 hours of light, and still more when given 15 hours

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR TWO HOURS AT −10° C. INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

DAY-LENGTH IN GREENHOUSE	HOURS OF LIGHT IN HARDENING ROOM	DAYS OF HARDENING TREATMENT			
		0	3	7	21
Short	0	382	393	384	386
	7	382	262	222	113
	14	382	218	174	89
Long	0	447	446	431	387
	7	447	315	317	171
	14	447	307	224	138

of light each day. In the dark, the short-day plants hardened not at all; the long-day plants hardened very slowly, but perceptibly.

Since it was clear at the end of the first week that but little hardening was going on in the plants in the dark, it seemed desirable to determine the result when plants were given light, but not carbon dioxide. Two pots were taken from the long-day (greenhouse) set which had been hardening in the dark for 10 days. One pot was put under a bell-jar which was sealed with sodium hydroxide solution on the bottom; free access to air was provided through a soda-lime tube at the top. Paper soaked in sodium hydroxide solution was stuck to a portion of the side wall of the bell-jar to take up any carbon dioxide set free in respiration. The other pot was similarly covered with a bell-jar which was sealed with water at the bottom. To this latter jar a carbon dioxide generator was connected each morning. Phosphoric acid was placed in the generator flask, and 100 cc. of M/50 sodium carbonate were allowed to flow slowly, through a capillary tube, into the acid. This slowly set free the carbon dioxide for a period of about three hours, it flowing through the delivery tube into the bell-jar. In this way, between 40 and 50 cc. of carbon dioxide gas were supplied to the pot each day. The bell-jars were placed side by side at 0° C., and were illuminated for 15 hours each day. This hardening period lasted for 12 days, when samples were prepared and treated as usual. Table III shows the results of determinations of electrical conductivity with duplicate samples from three pots, of which one was in the dark during this period, the second in the light without carbon dioxide, and the third in the light with carbon dioxide.

TABLE III

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR TWO HOURS AT −10° C. INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

CONDITION DURING HARDENING	SPECIFIC CONDUCTIVITY
No illumination, 0° C.	387
Illuminated 15 hours each day, 0° C., no CO_2	394
Illuminated 15 hours each day, 0° C., given CO_2	222

The figures in table III show that hardening proceeded almost identically in the cases of plants in the dark and those illuminated, but without carbon dioxide in the surrounding atmosphere. The plants which received carbon dioxide hardened in the usual way. This seems to indicate that hardening is definitely associated, in these rather succulent tissues, with photosynthesis, and that the influence of light at this temperature may be largely due to carbohydrate synthesis.

At the end of the hardening treatment, two pots of short-day (greenhouse) plants, one from the dark, the other from the long-period illumination treatment, were harvested for the determination of dry matter. The hardened plants (from the light) were much higher in dry matter (20.8 as compared with 13.0 per cent.) than those which had not hardened (in the dark). Curiously, however, the total amount of dry matter in the hardened plants had not increased in any such proportion. There seemed, therefore, to have been a dehydration process going on in the hardening plants, in the light, which increased the percentage of dry matter by loss of water as well as by gain in carbohydrates. Further study may determine whether or not this is actually the case.

REPETITIONS OF EXPERIMENT

Experiment 2 was repeated, with variations, with plants of three varieties of winter wheat grown out-of-doors until five weeks of age (September 23 to October 27). When these plants were given hardening treatment at 0° C. in the dark, with 7 hours and with 15 hours of illumination each day, for a period of 28 days, hardening was least complete in each case in darkness, more complete with 7 hours, and most complete with 15 hours of illumination. The average (for the three varieties) conductivity value at the beginning of hardening treatment was 884; in the dark this value was 398 at the end of the hardening period. With 7 hours of illumination each day the value was 292; and with 15 hours, 237. These plants, which were much more sturdy and higher in dry matter than the plants grown in the

greenhouse, hardened considerably in the dark, whereas the succulent greenhouse plants did not. The alfalfa plants which were well stored with reserve foods (experiment 1) also hardened somewhat in the dark. In each case, however, hardening was more complete in the light.

The plants of the three varieties of wheat were also given 7 and 15 hours of illumination each day at 10° C., for 28 days. These plants, while hardening slightly at first, became very chlorotic and more tender as the experiment proceeded. As in the case of the alfalfa plants, those receiving the longer day at this higher temperature were more tender than those receiving the 7-hour day.

Experiment 3

In this experiment, the effect of the presence or absence of carbon dioxide in the air was studied at different temperatures and with four species of plants. Minhardi wheat, hardy alfalfa, cabbage, and tomato plants were used. The wheat, cabbage, and tomato plants were grown in sand culture with nutrient solutions, and were five weeks old at the beginning of the hardening treatment. The plants were very uniform in appearance. The alfalfa plants were nine weeks old when hardening treatment began. They were grown in soil and were somewhat irregular in appearance. The wheat received a short day (8 hours) in the greenhouse, while the other plants received a long day (about 14 hours). The photographs show the size of the plants. There were 30 wheat plants, approximately 30 alfalfa plants, 8 tomato plants, and 8 cabbage plants, respectively, in each can. Samples were prepared as follows. The wheat samples were prepared as in experiment 2, using duplicate 1-gram samples. The alfalfa root samples were prepared by removing the leaves at the crown, washing in distilled water, and drying the surface water. A single 0.5-gram sample (about 25 roots) was used. The tomato and cabbage samples were prepared by removing the roots, and washing the tops and drying between towels. Duplicate 3-gram samples of the tops of each were used. The wheat and alfalfa samples were placed in test-tubes and were frozen in an alcohol-ice slush at $-8°$ C. for 2 hours; they were thawed in the water-bath at 2° C. for 30 minutes. To each tube in turn, 10 cc. of distilled water at 2° C. were added. Exosmosis continued for 16 hours at 2° C., when the electrical conductivity was measured at that temperature. The cabbage and tomato samples were placed in 1×8-inch test-tubes, frozen at $-5°$ C. for 4 hours, thawed as indicated, and 25 cc. of distilled water at 2° C. added to each tube. Conductivity measurements were made after 14 hours of exosmosis. There is generally considerable super-cooling without freezing when plant tissues are subjected to a temperature no colder than $-5°$ C. This experimental difficulty was met with regularly when this temperature was used. If,

however, the tissues were moved about in the tube with a clean glass rod after they had been thoroughly chilled, or if the tube was sharply rapped against a solid body, freezing generally occurred rather promptly. In some cases, especially when the plants were partially hardened, it was necessary to start crystallization by placing the tubes for a moment or two in a colder bath (−7 or −8° C.), after which they were replaced in the −5° C. bath where freezing always continued without difficulty.

The plants in this experiment were hardened in the following way. Two glass-walled chambers, about 8 cubic feet each, were placed in the cold room at 0° C. Into each chamber a tube of air was led from a compressed air line. In the case of one chamber, the air was merely dehydrated (with sulphuric acid and glass wool); in the other chamber, the dried air was passed through a long tube filled with alternate layers of soda-lime, glass wool, and solid sodium hydroxide, to remove the carbon dioxide from the air. Since the chambers were by no means air-tight, and had to be opened to water the plants, etc., doubtless there was some carbon dioxide in the chamber in any case. To avoid this as much as possible, further sodium hydroxide solution was placed within the case. Into each case eight cans of plants were placed, that is, two cans of each of the four species. Both cases received light from a 1000-watt lamp for 14 hours each day. During illumination the temperature in the cases went up to about 2° or 3° C.

In a warmer room, at about 20° C., two similar cases were arranged, one with ordinary air and the other with carbon dioxide-free air. These plants

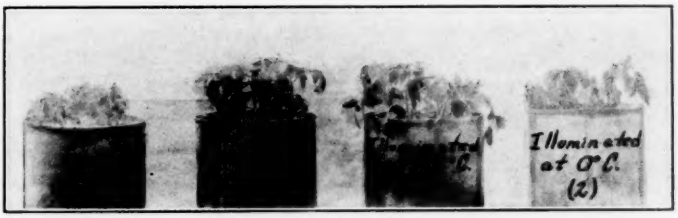

FIG. 3. Condition of tomato plants from experiments 3 and 4 after 2 days of treatment. On extreme left is the pot of plants which received no carbon dioxide; next to it, the one which received carbon dioxide. The plants receiving no carbon dioxide suffered very severe injury in the cold room, although they did not freeze; the others were uninjured. The two pots on the right are from experiment 4. The plants in the pot on the extreme right received illumination for four periods of 2 hours each at 0° C. with the other 16 hours each day at 22° C. They were badly injured by the cold. The other plants received an equal illumination, but at 22° C., with the remaining 16 hours at 0° C. They were uninjured by the cold. In this case, an opportunity for high photosynthesis and low respiration may well explain their ability to survive the cold for 16 hours, whereas the plants with an opportunity for high respiration and low photosynthesis could not survive.

received 14 hours of illumination each day at a temperature of approximately 24° C. Each day, at the end of the illumination period, the plants from both of the glass cases in the warm room were transferred to the cold room, where they remained for 10 hours in the dark at 0° C. They were then transferred back to the cases in the warm room, and the process was repeated. At the end of two days the photograph shown as figure 3 was taken. This shows that the tomato plants receiving light in the warm room, but no carbon dioxide, were very badly injured in the cold room, although they were not frozen. This injury was plainly evident after the first night in the cold room, following the first day without carbon dioxide. The plants receiving carbon dioxide were not injured perceptibly by the exposure in the cold room.

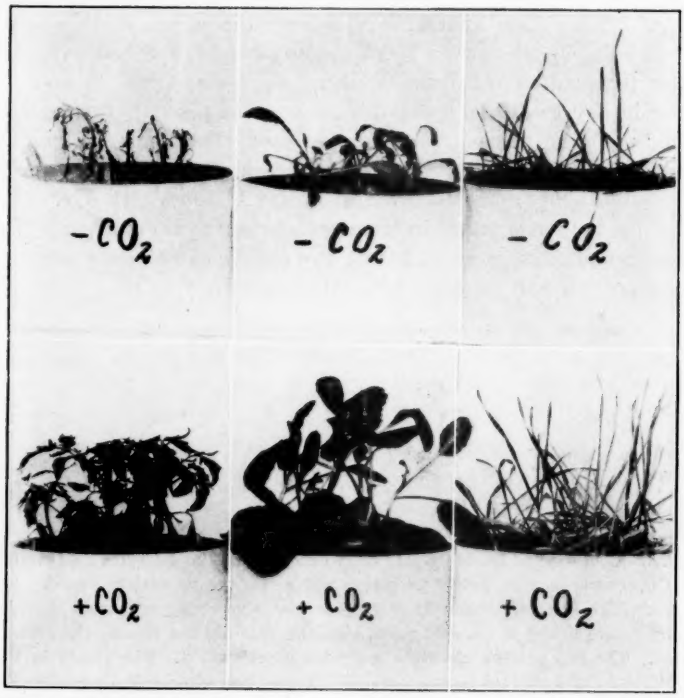

FIG. 4. Condition of the two sets of plants in the room at about 24° C. at the end of two weeks of treatment. Each set received 14 hours of illumination in the warm room, and 10 hours in the dark at 0° C. each day. The upper set received carbon dioxide-free air during the two weeks, and the plants were almost dead. The lower set received ordinary (dehydrated) air and were in good condition.

The other pots in figure 3 received an alternating temperature and light treatment as described in experiment 4. The uninjured plants received their light (four 2-hour periods of illumination) in a warm room, while the badly wilted plants next to them, on the extreme right, received equal illumination, but in the cold room. Again the injury was plainly evident after one day of treatment.

Photographs of the plants with and without carbon dioxide in the warm room, taken at the end of two weeks, are shown in figure 4. Here it is evident that the cabbage and tomato plants receiving light but no carbon dioxide were almost dead, and the wheat was wilted and yellow. The pots of alfalfa plants were omitted from the photograph, since the numbers of plants per pot were not identical. The alfalfa plants showed the starvation effect later than the other plants, but it was evident at the end of two weeks.

At the end of one week of hardening treatment, and at the end of two

TABLE IV

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF SAMPLES OF THE PLANTS IN EXPERIMENT 3. SAMPLES OF WHEAT AND ALFALFA WERE FROZEN FOR 2 HOURS AT − 8° C.; THOSE OF CABBAGE AND TOMATO FOR 4 HOURS AT − 5° C.; FOURTEEN HOURS OF ILLUMINATION DAILY

SPECIES	CARBON DIOXIDE	DAYS OF HARDENING TREATMENT			REMARKS
		0	7	14	
Continuously in cold at 0° C.					
Alfalfa	Present	297	274	210	
Alfalfa	Absent	297	314	317	
Wheat	Present	650	367	226	
Wheat	Absent	650	499	345	
Cabbage	Present	804	658	486	
Cabbage	Absent	804	1027	1445	
Tomato	Present	1060	1006	1250	Both sets almost dead
Tomato	Absent	1060	1159	1385	
Illuminated at 24° C., and 10 hours in dark at 0° C.					
Alfalfa	Present	297	263	296	
Alfalfa	Absent	297	292	320	
Wheat	Present	650	567	621	Decided elongation in both cases
Wheat	Absent	650	635	700	
Cabbage	Present	804	976	707	Approximately doubled in size
Cabbage	Absent	804	1093	1709	Almost dead
Tomato	Present	1060	1052	946	Approximately doubled in size
Tomato	Absent	1060	1081	1385	Almost dead

weeks, samples from the four sets of plants were prepared for testing, as previously described. The conductivity determinations are given in table IV.

From the figures in table IV, it is evident that hardening was favored by the presence of carbon dioxide in the air. The different reactions of the four species are of considerable interest. Thus wheat and alfalfa, and to a less extent cabbage, hardened much more thoroughly when kept continuously in the cold. The tomato plants, however, were severely injured by the conditions of hardening before the period was over, with the exception of the plants receiving carbon dioxide and light at the higher temperature. The tomatoes would not survive the occasional exposures to a temperature of 0° C. unless given illumination at a higher temperature for a part of the day. Experiment 4, which follows, brings this point out further. It is also of interest to note that, of the plants in the cold room continuously, but largely without carbon dioxide, the winter wheat alone hardened. Wheat plants of the same set in the same room in the dark showed no hardening whatsoever. In experiment 2 it was shown that when carbon dioxide was completely excluded, wheat hardened almost precisely the same in the light as in the dark.

As the photographs in figure 4 show, the tomato and cabbage plants grown in the warm room, with carbon dioxide, grew extensively in the two weeks' period, and almost doubled their green weight. This was, assuredly, unfavorable for hardening. It may well be also that the long day was a further aggravating tendency. The work of HARVEY (**4**) would indicate that a temperature of from 5° to 10° C. lower might have caused the cabbage plants to harden much more completely under the conditions of alternating temperatures; in fact, fully as completely as those kept continuously in the cold room. His photographs indicate that there was no perceptible difference in the size of the plants hardened with constant low, and alternating high-low temperatures. This difference in the growth behavior of the plants would readily account for the difference in hardening response in the two experiments.

The winter wheat plants which received a long day at 24° C. elongated their leaf sheaths very notably, whether they received carbon dioxide or not. The plants receiving no carbon dioxide, however, were much more chlorotic.

Experiment 4

In this experiment, four species of plants were again used, and of the same kinds and ages. However, the alfalfa was of another variety, and the cabbage and tomatoes were grown in soil. Only the wheat samples were identical with those used in experiment 3. The samples were pre-

pared as before, except that 4-gram duplicate samples of the tomatoes and cabbage were used. Freezing and other details were carried out as described for experiment 3.

In this experiment, an attempt was made to provide conditions during hardening which would combine the effects of alternating temperatures and "cold shocks" with those of increased photosynthesis or respiration. The plants were hardened in five ways: (1) Illuminated for 2 hours at 22° C., then moved into the dark in the cold room at 0° C. for 2 hours, again into the light in the warm room; repeating four times. Thus the plants received 8 hours of interrupted illumination in the warm room, with a total of 16 hours each day in the dark at 0° C. (2) Precisely as the previous case, except reversed; that is, illumination was given only in the cold room, darkness only in the warm room. There was, therefore, 8 hours of interrupted illumination at 0° C. and 16 hours in the dark at 22° C. (3) These plants received the same temperature treatment as (1) but no light; that is, 8 hours, interrupted, in the room at 22° C. and 16 hours at 0° C., all in the dark. (4) Continuous dark, at 0° C. (5) Continuous light at 0° C.

In no case were the plants which were moved kept in absolute darkness; they were covered with heavy cardboard boxes, but could receive a fraction of a foot-candle of illumination when presumably in darkness.

Figure 3 shows the sharp difference in behavior in the tomato plants of treatments 1 and 2, photographed at the end of the second day. From this reaction it would appear that the alternating temperature behavior might well be associated with increased photosynthesis and reduced respiration in treatment 1 as contrasted with slow photosynthesis and high respiration in treatment 2. Figure 5 shows the plants from treatments 1, 2, and 3 at the end of 13 days of hardening treatment. Again the pots of alfalfa plants are omitted. (During the second week, there were two periods of illumination of 4 hours each, rather than four periods of 2 hours each.) When the photographs were taken, the tomato and cabbage plants of treatment 1 had made considerable vegetative elongation; those of condition 3 (alternating temperature in darkness) were almost dead; and the tomatoes in treatment 2 (light given at 0° C.) were also almost dead. In the two remaining treatments, 4 and 5, none of the plants made apparent growth, while the tomato plants gradually died, although those receiving light survived longer than those in darkness. Of the five treatments, but one set of tomato plants survived the experiment, namely, treatment 1. This was precisely the result found in the experiment previously described, in which it was shown that the tomato plants appeared to require an opportunity for photosynthesis at a fairly high temperature if they were to survive occasional exposures to a temperature of 0° C.

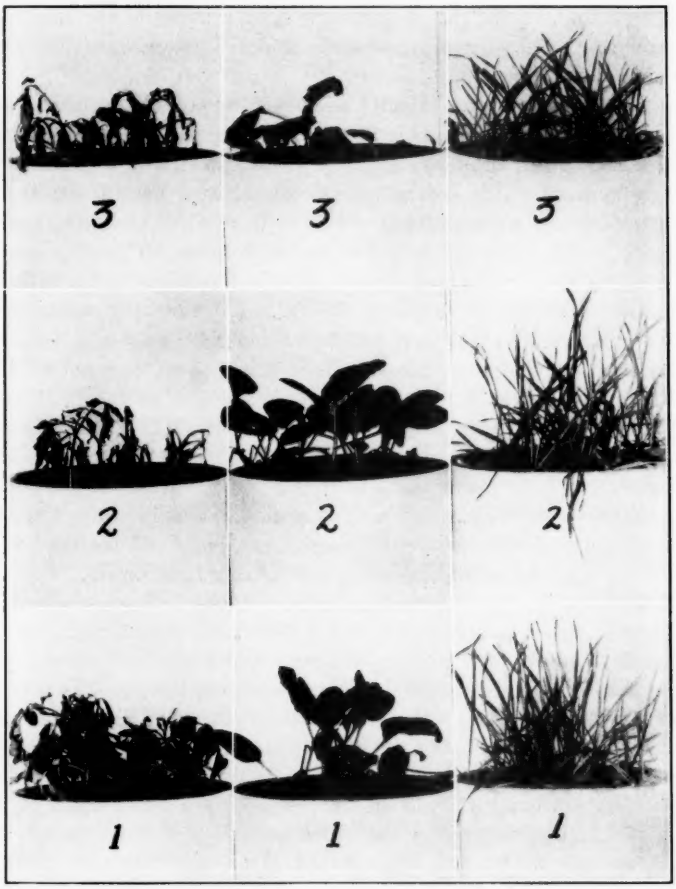

FIG. 5. Condition of the plants after 13 days of treatment. The top row of pots received alternating temperatures in the dark (treatment 3); the middle row received light in a cold room (0° C.) in four 2-hour periods, and the remaining 16 hours in the dark at about 22° C.; the lower row received light in a room at about 22° C. in four 2-hour periods, and the remaining 16 hours in the dark at 0° C. The tomatoes in the bottom row were almost uninjured, whereas the others were severely injured. The cabbage plants in the top row were almost dead. All sets of wheat were adversely affected by the warm temperature treatment, but those of the top row were in a very chlorotic condition and almost dead.

The leaves of the cabbage plants from treatment 3 were so badly wilted and shrunken that it was impossible to get strictly comparable samples, but the conductivity value is included in the table. Comparable samples of the

tomato plants could not be obtained, since, in all except treatment 1, only stumps of the stems remained alive. Table V gives the conductivity values of the samples at the beginning of the trial, and after two weeks of hardening treatment.

TABLE V

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2°C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS FROM FROZEN PLANTS IN EXPERIMENT 4; FREEZING TREATMENT AS IN EXPERIMENT 3

PLANT	TREATMENT	DAYS OF HARDENING TREATMENT	
		0	14
Alfalfa	1. Warm light-cold dark	388	265
	2. Cold light-warm dark	388	263
	3. Continuous dark, warm-cold	388	321
	4. Continuous dark and cold	388	289
	5. Continuous light and cold	388	265
Wheat	1. Warm light-cold dark	650	500
	2. Cold light-warm dark	650	633
	3. Continuous dark, warm-cold	650	616
	4. Continuous dark and cold	650	659
	5. Continuous light and cold	650	234
Cabbage	1. Warm light-cold dark	1085	702
	2. Cold light-warm dark	1085	751
	3. Continuous dark, warm-cold	1085	1750
	4. Continuous dark and cold	1085	1047
	5. Continuous light and cold	1085	463
Tomato	All very severely injured in hardening process except (1), warm light-cold dark, which survived very well. No comparable samples obtainable		

Table V shows that hardening was more complete in treatment 1 than in treatment 2. In the case of the alfalfa, the roots were buried in the soil, of course, and did not actually receive the sharply alternating temperatures that the other parts tested did. The wheat and cabbage gave evidence of more complete hardening in 1 and in 2 by their conductivity values; the tomatoes by their survival at the temperatures used (figs. 3, 5). There seems to be no evidence that alternating temperatures as such (treatment 3) were beneficial in hardening these plants. In fact, on the average this would seem to have been the poorest treatment of the five used in this experiment. This seems not unreasonable, since it gave a considerable opportunity for respiration and no opportunity for photosynthesis, except during the intervals when the plants were being moved, or in the slight illumination under the covers. By all means the most efficient hardening

treatment (with the exception of the tomatoes) was the continuous light with continuous cold. Tomatoes, however, did not seem to be able to endure this temperature without severe injury, unless receiving illumination at a higher temperature for part of the day. It seems probable that photosynthesis, with the tomato, is almost negligible at the temperature near 0° C.

Discussion and conclusions

The results of these experiments all point, in the opinion of the writer, to similar conclusions. The general proposition may be stated that hardening of plants is favored by conditions which tend toward the accumulation or conservation of carbohydrates and other reserve foods; that is, which further photosynthesis and lessen respiration and extension of vegetative parts. Hardening proceeded markedly in the dark in alfalfa or winter wheat plants that had an abundant storage of organic food, if the temperature was near 0° C. The more succulent wheat plants, grown in the greenhouse, gave no indication of hardening in the dark at 0° C., but hardened rather completely when illuminated. Alternating temperatures were not shown to be particularly favorable to hardening in either light or dark. A short period, with light, at a higher temperature was not especially deterrent in the hardening process, except in the case of winter wheat. This cannot be said of a longer day at the higher temperature, for in this case, with both alfalfa and wheat, and perhaps with cabbage and tomato, elongation of foliar parts was especially evident. The species are by no means identical, however, in their reactions. Winter wheat was much more adversely affected in its hardening behavior than alfalfa, cabbage, or tomato by a period of short duration at a higher temperature. In fact, tomato plants could not survive occasional exposures to a temperature of 0° C. unless they had opportunity for photosynthesis at higher temperatures.

Exposure to a long day in the hardening room at 0° C. gave no indication of the usual response in wheat and alfalfa of decided elongation of parts. On the contrary, the plants hardened more fully with such long-day illumination treatment than with a short day. Winter wheat plants which had received a short day in the greenhouse (60° F.) hardened more rapidly and more fully in the cold room than similar long-day (greenhouse) plants under either a long- or a short-day hardening treatment. The short-day plants were smaller, greener, and somewhat higher in dry matter.

Further evidence of the relation of organic foods to hardening is given by experiments 3 and 4. Winter wheat, alfalfa, tomato, and cabbage plants were hardened in several ways which would favor photosynthesis

and decrease respiration, or the reverse. Removal of carbon dioxide from the air given the plants in all cases prevented or greatly depressed the degree of hardening. Plants receiving their light in a warm room and their dark period in a cold room were in all cases hardier than those receiving the opposite treatment. The first would seem to favor photosynthesis and depress respiration, while the second would tend to use the reserve foods of the plant without replenishment. Alternating temperatures, as such, without illumination did not appear to be helpful in the hardening of the plants. When marked top growth occurred, even under conditions favoring photosynthesis and depression of respiration, hardening was lessened.

Although the various species used differed considerably in their behavior, the general suggestion seems to hold that the development and maintenance of a high available carbohydrate supply, with much retarded vegetative growth, is essential before the cold-temperature reaction of hardening of plants will occur in an efficient manner.

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INFLUENCE OF VARIOUS NITROGENOUS COMPOUNDS AND MANNITOL ON NODULE FORMATION BY CLOVER[1]

E. W. Hopkins and E. B. Fred

(with two figures)

Introduction

In a previous paper (**9**), the influence of nitrate upon the number and distribution of nodules on red clover plants was considered. It seemed desirable to continue this work, using a number of organic nitrogenous compounds.

The wide occurrence of organic nitrogenous compounds in soil makes it imperative that these compounds as well as nitrates be considered in their effect upon nodule formation. Investigations on this problem have been limited. Frank (**4**) found that peas fixed more nitrogen when urea was added to sand cultures, and that lupines behaved in an opposite manner. Flamand (**2**) determined that the following concentrations of urea and oxamide inhibited nodule formation in Sach's solution: *Pisum sativum*, urea less than 1/20,000, oxamide 1/20,000; *Vicia narbonensis*, urea and oxamide 1/20,000; *Faba equina*, urea 1/20,000, and oxamide 1/10,000. Similarly, the carbonaceous materials of soil may play a rôle in nodulation. This problem has also received little attention. Ritter (**14**) reported that in soil, sucrose had no effect on the formation of nodules by lupines. Prucha (**13**), however, found that more than 2 gm. of sucrose added to 300 gm. of soil adversely affected nodulation of peas, while 0.2 to 2 gm. of sucrose apparently had no influence. According to Wilson (**18**), maltose, fructose, lactose, sucrose, and glycerol stimulated nodule formation on soy beans, while glucose was inactive. Calcium saccharate was found to possess a particularly stimulating effect.

Experimentation

In the following study, the attempt was made to produce a plant environment such that the nodule bacteria would not find invasion of the plant roots necessary in order to obtain carbohydrate and fixed nitrogen. Although such environments modified nodule formation, in no case was actual inhibition of nodules observed.

The nitrogen compounds added to the plant cultures were KNO_3, $(NH_4)_2SO_4$, urea, asparagin, clover-seed extract, and yeast extract. In a second series, mannitol was added in addition to the nitrogen compounds.

[1] Herman Frasch Foundation in Agricultural Chemistry, Paper no. 29. Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

METHODS.—The methods and details of planting are given in the previous paper (**9**). In the tests where mannitol was added, the concentration used was 0.5 per cent. To avoid decomposition of the nitrogenous compounds during sterilization, these were added with aseptic technique to the sterile agar shortly before hardening occurred. Sterilization of the solutions of KNO_3, $(NH_4)_2SO_4$, urea, and asparagin before addition to the agar was effected by filtration through a Berkefeld filter. The asparagin solution was neutralized with NaOH to the phenolphthalein end-point. The yeast and clover-seed extracts could not be filtered, and so were sterilized in the autoclave. Solutions of the nitrogen sources were added in such amounts to the bottles that series of 2, 5, 10, and 20 mg. N per bottle were obtained.

The yeast extract was made up as given by FRED and WAKSMAN (**5**). In the series with no mannitol added, the clover seed extract was prepared by soaking the seed and then steaming. For the mannitol series, the extract was obtained from macerated clover seedlings. The extracts were filtered through paper pulp. The nitrogen present in these extracts was determind by the Kjeldahl-Gunning method.

PLANT GROWTH.—The large number of bottles required for the experiment made it necessary to extend the planting over a period of several weeks. Ten bottles of each treatment were included; thus a total of over 500 bottles was required. At the time of harvest, the difference in age of the cultures was about one month. This difference in age, however, would not appear to be an important consideration, since at the time of harvest all plants had attained the maximum development possible under the conditions. The cultures ranged in age from 90 to 120 days. The growth period was during November, December, January, and to the middle of February. During this period, plant growth is very slow owing to deficient light. The plants were exposed to artificial illumination, and changed about to maintain conditions as constant as possible throughout the growth period.

At the end of the experiment, the plants were pulled from the agar. There was very little loss of root material, because the agar was soft enough to allow passage of the roots without breaking them. The plants were examined for their general appearance, and nodule counts were made. The size, shape, and distribution of the nodules were noted. Plants from three bottles were combined, the total nitrogen determined by the Kjeldahl-Gunning method, and the results calculated for ten plants.

The effects of the various compounds upon the growth of the plants will be considered under the discussion of each nitrogen source, and the effect on nodule formation in the general discussion.

CONTROLS.—The growth of the controls containing no mannitol was very good. At all times the plants possessed a deep green color, and at the time

of harvest, filled the bottles. In the controls containing mannitol, growth was somewhat slower, but the general condition of the plants and their color was good; table I gives the data for the two sets of controls.

TABLE I

EFFECT OF MANNITOL ON NODULE FORMATION

TREATMENT	TOTAL N IN 10 PLANTS (1 BOTTLE)	NUMBER OF SAMPLES	NODULE MEAN, 10 PLANTS	DISTRIBUTION AND SIZE OF NODULES
	mg.			
Uninoculated control	2.3	...	.....	.....
Control 1: no mannitol	9.7	9	138 ± 11	Nodules principally on tap-roots, many near crown, mostly of the round type; roots normal
Control 2: 0.5 per cent. mannitol	9.4	9	95 ± 6	Nodules principally on tap-roots, many near crown, mostly of the long type and very large; roots slightly stunted in a few plants

INORGANIC NITROGEN COMPOUND SERIES.—In these series, KNO_3 and $(NH_4)_2SO_4$ were present in varying amounts. The plants grew very well in the nitrate series without mannitol, and in color and size were even better than the controls. In the mannitol series, with increasing nitrate, the plants did not grow well. In this series, as in all others containing mannitol, the organisms grew over the surface of the agar and along the plant roots, increasing vigor of growth of bacteria being evidenced in the higher concentrations of the nitrogen source. This growth apparently was unfavorable for the plants, because as the amount of growth increased, the roots became more stunted, and frequently were gnarled and very irregular, or failed to put out secondary roots. In some cases, however, the plants were able to attain good growth in spite of this condition. None of the concentrations of nitrates used was found to prevent bacterial growth, so it is probable that the organisms were not injured by this salt, as HILTNER

TABLE II

EFFECT OF INORGANIC NITROGEN COMPOUNDS ON NODULE FORMATION

NITROGEN ADDED PER BOTTLE	TOTAL N IN 10 PLANTS (1 BOTTLE)	NO. OF SAMPLES	NODULE MEAN, 10 PLANTS	CRITICAL RATIO, STANDARD DEVIATIONS	DISTRIBUTION AND SIZE OF NODULES
mg./250 cc.	*mg.*				
Uninoculated control	2.3	.....	.....	.....	.....
Series 1: KNO_3, no mannitol					
2	10.4	9	152 ± 8	+1.03	Nodules present principally on secondary roots, largely of round type, some distributed over root system, decreasing in size with increasing nitrate; roots normal
5	9.0	10	161 ± 11	+1.47	
10	11.1	10	130 ± 13	−0.41	
20	11.4	11	110 ± 12	−1.72	
Series 2: KNO_3, 0.5 per cent. mannitol					
2	8.5	9	90 ± 11	−0.40	Nodules principally on tap-root, many near crown, large, mostly of long type, decreasing in size with increasing nitrate; roots stunted with increasing nitrate
5	9.3	10	80 ± 5	−1.92	
10	6.4	9	55 ± 4	−5.32	
20	5.0	.....	.....	.....	
Series 3: $(NH_4)_2SO_4$, no mannitol					
2	6.2	10	64 ± 5	−6.15	Nodules principally on secondary roots, mostly of round type but some long, decreasing in size with increasing ammonia; roots slightly stunted
5	6.8	10	70 ± 8	−5.00	
Series 4: $(NH_4)_2SO_4$, 0.5 per cent. mannitol					
2	3.9	10	50 ± 6	−5.30	Nodules principally on tap-root, in 2 mg., many near crown, largely of long type, many large round type increasing with increasing ammonia; roots stunted with increasing ammonia
5	5.6	7	43 ± 5	−6.65	
10	5.2	.....	.....	.....	
20	3.1	.....	.....	.....	

(**8**) has assumed. The other nitrogen sources appeared to be equally as good or better than nitrate in producing growth of the organism. Thus no toxic effect on the organism was observed with any nitrogen compound used.

Bacterial growth had covered the roots of the plants in the 20-mg. set, with the result that the plants were badly stunted. The set was discarded.

In the $(NH_4)_2SO_4$ series, without mannitol, all the plants in the 20 mg. of NH_3-N set and most of those in the 10-mg. set died. When mannitol was present, none of the plants was killed; but when harvested, they were so small that nodule counts were not made. In the lower concentrations of ammonia, in both the mannitol and non-mannitol series, the plants attained a fair growth but were of a pale green color. Table II gives the data of the KNO_3 and $(NH_4)_2SO_4$ series.

ORGANIC NITROGEN COMPOUND SERIES.—In these series, urea and asparagin (as the sodium salt) were added to the agar. In both urea series, with and without mannitol, the plants attained a good size, and were dark green in color. The higher concentrations of urea, however, were toxic to the plants, and in the series without mannitol the plants died in the 20 mg. of urea-N set. The plants in the 10-mg. set developed rather slowly at first, but after slow growth at the start, were able to outgrow the other series. In the urea series with mannitol, all sets above 5 mg. of urea-N grew very poorly, and at the end of the experiment the plants were only a few cm. high.

In the asparagin series excellent plant growth was obtained in most of the sets. The plants in the 20 mg. of asparagin-N died, but the color of the plants in all sets was even darker than that of the controls. This series is especially interesting because some workers have considered amides to be the first compounds formed in the fixation of nitrogen by leguminous plants. FRANK (**3**) expressed the view that nitrogen was fixed as amides in the leaves of the Leguminosae. STOKLASA (**15**) believed that amides were the first compounds formed in nitrogen fixation. It is somewhat surprising that the concentration of 20 mg. of asparagin-N would be high enough to kill the plants, especially since the nitrogen present was only about one-quarter to one-half more than ten plants would require for their growth under the conditions used. When mannitol was present with asparagin, poor plant growth took place in the higher concentrations.

The excellent growth obtained in series 7 is no doubt due to the utilization of asparagin by the plants. VIRTANEN and VON HAUSEN (**17**) have recently shown that aspartic acid and the amino acids in a casein digest are readily assimilated by red clover. It is very probable, they believe, that free nitrogen is fixed in the nodules as amino acids.

The data for the urea and asparagin series are given in table III.

TABLE III

EFFECT OF ASPARAGIN AND UREA ON NODULE FORMATION

NITROGEN ADDED PER BOTTLE	TOTAL N IN 10 PLANTS (1 BOTTLE)	NO. OF SAMPLES	NODULE MEAN, 10 PLANTS	CRITICAL RATIO, STANDARD DEVIATIONS	DISTRIBUTION AND SIZE OF NODULES
mg./250 cc.	*mg.*				
Uninoculated control	2.3	.....	.....	.....	.....
Series 5: urea, no mannitol					
2	9.3	10	111 ± 10	−1.81	Nodules principally on secondary roots, some distributed over root system, largely of round type, decreasing in size with increasing urea; roots normal
5	9.0	10	110 ± 9	−1.95	
10	9.5	11	51 ± 7	−6.70	
Series 6: urea, 0.5 per cent. mannitol					
2	7.9	10	58 ± 5	−4.73	Nodules principally on tap-root, mostly round type, though many long type, decreasing in size with increasing urea; roots stunted with increasing urea
5	5.5	9	62 ± 7	−3.67	
Series 7: asparagin, no mannitol					
2	9.8	11	106 ± 8	−2.35	Nodules principally on secondary roots, largely of round type, increasing distribution along root system with increasing asparagin; roots normal
5	10.8	10	100 ± 9	−2.68	
10	14.2	10	111 ± 15	−1.45	
Series 8: asparagin, 0.5 per cent. mannitol					
2	6.9	10	72 ± 5	−3.58	Nodules principally on tap-root, many near crown, principally of long type, changing to round type with increasing asparagin; roots stunted with increasing asparagin
5	7.2	10	128 ± 20	+1.58	
10	6.4	10	53 ± 9	−3.89	
20	6.7	.....	.....	.....	

PLANT EXTRACT SERIES.—To these series, clover-seed extract and yeast extract were added to the agar. With both of these nitrogen sources, plants in the non-mannitol series attained a moderate growth, but were of a rather pale color. In the mannitol series, all plants grew very well and possessed a dark green color. In appearance, the plants were better than those of any other series. Table IV gives the data on the clover-seed and yeast extract series.

Discussion

The data in these experiments may be discussed according to the nitrogen compounds used. In another part of this paper, the results will be considered as a whole.

For the mean of the number of nodules in each set, the standard deviation was computed, and the effectiveness of the treatment determined by the method of the critical ratio. This was calculated by comparing each set with its control. A critical ratio of 2.5 standard deviations was taken as the limit of significance.

CONTROLS.—The data are given in table I. The amount of nitrogen contained in the plants in these sets will be used as the comparison for that of the sets receiving nitrogen compounds. Mannitol decreases the number of nodules which form, but these nodules are larger than those of plants without mannitol. Other workers have commented on the increased size of nodules produced by carbohydrates. HARRISON and BARLOW (**7**), growing pea and vetch in 0.4 per cent. maltose-wood ash agar, observed that very large nodules were produced. LEONARD (**11**) found that when glucose was added to sand cultures of soy beans in bottles, the roots were injured, although the nodules which formed were twice the normal size. The nodules in the controls are located principally on the tap-root, with many close to the crown. In the controls without mannitol, the round nodules predominated, while in those with mannitol present, the long type were more numerous. The statistical constant was not calculated for the controls.

INORGANIC NITROGEN COMPOUNDS SERIES.—Comparing the nitrogen content of series 1 and 2 (table II), it is apparent that the plants with mannitol assimilated less nitrogen than those without. The heavy growth of bacteria which occurred on the surface of the agar and along the plant roots of the mannitol-nitrate set apparently exerted a toxic effect upon the plants. The number of nodules is decreased significantly only in the 10-mg. set in series 2. With all of the nitrogen present at the start, it was found in a previous experiment (**9**) that the number of nodules was irregularly affected. The distribution of the nodules in series 1 is affected as noted previously (**9**); that is, that even a small amount of nitrate causes the nodules to form principally on the secondary roots, such nodules being of the small round

TABLE IV

EFFECT OF CLOVER-SEED AND YEAST EXTRACTS ON NODULE FORMATION

NITROGEN ADDED PER BOTTLE	TOTAL N IN 10 PLANTS (1 BOTTLE)	NO. OF SAMPLES	NODULE MEAN, 10 PLANTS	CRITICAL RATIO, STANDARD DEVIATIONS	DISTRIBUTION AND SIZE OF NODULES
mg./250 cc.	*mg.*				
Uninoculated control	2.3	.....	.....	.....	.....
Series 9: clover-seed extract, no mannitol					
2	6.0	10	84 ± 8	−4.00	Nodules principally on tap-root, changing to secondary root with increasing extract, mostly of long type, changing to round type, some very large; roots stunted in increasing extract
5	7.3	10	64 ± 5	−6.12	
10	8.3	10	60 ± 7	−6.00	
20	7.3	5	30 ± 5	−9.00	
Series 10: clover-seed extract, 0.5 per cent. mannitol					
2	5.6	9	61 ± 6	−4.01	Nodules principally on tap-root, many near crown, predominantly of long type, many very large; roots stunted with increasing extract
5	11.7	9	71 ± 6	−2.83	
10	10.7	9	65 ± 4	−4.16	
20	10.4	10	43 ± 4	−7.21	
Series 11: yeast extract, no mannitol					
2	7.9	10	87 ± 7	−3.53	Nodules principally on tap-root, changing to secondary root with increasing extract, mostly of long type, changing to round type, very small with increasing extract; roots very slightly stunted
5	8.3	10	108 ± 9	−2.04	
10	11.7	8	74 ± 6	−5.12	
20	13.0	4	54 ± 12	−5.16	
Series 12: yeast extract, 0.5 per cent. mannitol					
2	8.2	10	50 ± 5	−5.76	Nodules principally on tap-root, predominantly of long type, many large; roots stunted with increasing extract
5	8.4	8	44 ± 3	−7.60	
10	14.3	10	82 ± 7	−1.41	
20	13.0	9	43 ± 5	−6.65	

type. With increasing nitrate, the size of the nodules decreases. When mannitol is present, however (series 2), the distribution of the nodules is not affected by the nitrate, although the size of the nodules decreases in the higher nitrate sets. The placement of the nodules is essentially normal,—mostly on the tap-root and near the crown. Mannitol increases the size of the nodules.

In series 3 and 4 (table II) the effect of $(NH_4)_2SO_4$ on nodule formation is shown. The plants did not grow as well as those in series 1 and 2, and did not assimilate as much nitrogen. The number of nodules was significantly decreased by all concentrations of $(NH_4)_2SO_4$. The effect upon position of the nodules was the same as that produced by nitrates, scattered nodules in series 3, and nearly normal nodules in series 4 with mannitol present.

ORGANIC NITROGEN COMPOUNDS SERIES.—In both the urea and asparagin series, the plants without mannitol (series 5 and 7, table III) assimilated more nitrogen than those to which mannitol was added. The amount of nitrogen in ten plants increased as more of the nitrogen compound was available. Asparagin seemed to be an especially good source of nitrogen.

The number of nodules was irregularly affected by the nitrogen treatment. As judged by the critical ratio, the number of nodules was decreased in series 5 by 10 mg. of urea-N, in series 6 by 2 and 5 mg. of urea-N, in series 7 by 2 and 5 mg. of asparagin-N, but not by 10 mg.; and in series 8 by 2 and 10 mg. of asparagin-N, but not by 5 mg. Asparagin seems to be less consistent than any of the nitrogen sources used in the effect on nodule formation.

The distribution of the nodules on the roots was very similar to that in the KNO_3 series (series 1 and 2, table II). The nitrogen sources produced scattered round nodules, even in the lowest concentrations, with decreasing size of the nodules when more of the nitrogen source was present. When both nitrogen compound and mannitol were present, as seen in the KNO_3 series, the nodules were placed largely on the tap-roots and attained a good size. However, the size of the nodules decreased as the concentration of the nitrogen compound increased.

PLANT EXTRACT SERIES.—The plants in series 9–12 exhibited good growth in both the series with and those without mannitol. When mannitol was present in addition to the nitrogen sources, the highest nitrogen plants were produced.

The number of nodules showed certain irregularities, but was generally depressed by all concentrations of the plant extracts. In all clover-seed extract sets, 2, 5, 10, and 20 mg. N with and without mannitol, the number of nodules was decreased. In the yeast extract series (series 11 and 12), the 5-mg. yeast extract-N set without mannitol, and the 10-mg. set with

mannitol showed no significant effect on the number of nodules. All other concentrations of yeast extract effectively decreased nodule formation.

In the distribution of nodules, it will be noted that the plant extracts produced less deviation from normal placement than did the other nitrogen sources. In the 2-mg. sets, without mannitol, the distribution of the nodules was essentially normal; but as the concentration of the plant extracts increased, the distribution of nodules was affected the same as in the case of

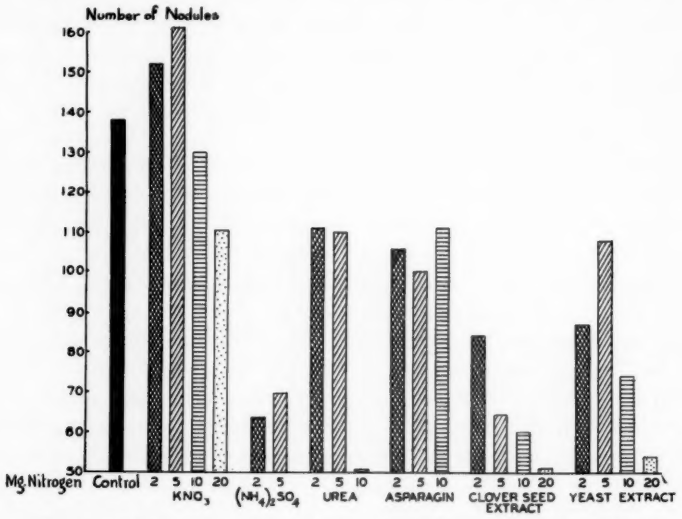

FIG. 1. Effect of various nitrogen compounds on nodule formation.

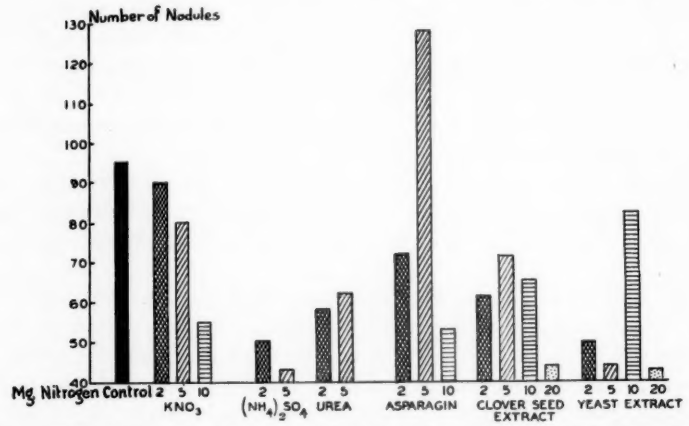

FIG. 2. Effect of various nitrogen compounds and mannitol on nodule formation.

the other nitrogen sources. Nodules then appeared principally on the secondary roots, and were small and round. When mannitol was present in addition to the plant extracts, no change from the normal placement of nodules occurred, but increasing concentrations of the plant extract resulted in smaller nodules.

Figure 1 shows the comparison of the nitrogen sources in the absence of mannitol, and figure 2, the series with mannitol and a nitrogen source.

From the foregoing data, certain generalizations may be made. When plants were grown in agar containing any one of the six nitrogenous compounds used, nodules on such plants were affected in two ways. All of the nitrogen sources were found, first, to decrease the size of the nodules formed, and second, to affect the distribution of the nodules on the roots. The number of nodules was influenced irregularly. Mannitol was found to have three effects: (1) to decrease the number of nodules, (2) to increase the size of the nodules which formed, and (3) to alter the position of the nodules on the roots.

The normal position of the nodules was near the crown of the root, with most of the nodules on the tap-root or very close to it on the secondary roots. In the normal plants there were a number of the long type of nodules, which are simply large nodules, while the largest number were of the round type, or small nodules. When the nitrogen sources were added, this distribution was changed as was noted for nitrate in a previous publication (**9**). Even in the lowest concentration (2 mg. of N) of KNO_3, $(NH_4)_2SO_4$, urea, and asparagin, the nodules were found, not principally on the tap-root but almost exclusively on the secondary roots. Yeast and clover-seed extracts did not noticeably change the distribution of nodules when 2 mg. of extract N were present, but in all higher concentrations the nodules formed on the secondary roots and were of decreased size. It is noteworthy that clover-seed extract influenced the size of nodules less than did any other nitrogen source.

Considering the series containing both mannitol and a nitrogen source, it will be observed that with mannitol present, the distribution of the nodules was more like that in control plants. While the nodules were fewer, many attained a very large size. The nodules were situated mostly on the tap-root, near the crown. It will be noted further that increasing amounts of the nitrogen source still decrease the size of nodules when mannitol is present, but do not change their position on the roots.

HILTNER (**8**) proposed that the plant was rendered immune to root invasion when nitrate was available. The view that nitrates are toxic to the nodule bacteria and thus influence nodule formation by injuring the inciting agent has been proposed. HILTNER believed that the nodule bacteria were injured in the soil, while STROWD (**16**) suggests that the high

concentration of nitrate attained in plant sap is sufficient to injure the invading nodule bacteria. LAURENT (**10**) expressed the view that nitrates and some constituent of the plant sap form a combination which is toxic to the nodule organisms. Experiments are offered to support this view.

GIÖBEL (**6**) has offered the ingenious theory that, since it appears probable that nitrogen is fixed in the nodules as an organic compound, such a compound would experience greater difficulty in diffusing through cell walls than would a rapidly transferable ion like NO_3. This condition would result in an accumulation in the nodule of the products of fixation, with the result that fixation of nitrogen would be impaired and development of nodules stopped.

MAZÉ (**12**) has considered the question from an entirely different point of view. His conception relates root invasion to the carbohydrates present in the plant sap. When the plant is receiving nitrate the carbohydrate may be assimilated at once, so that the sap of plants with adequate nitrogen supply would be low in carbohydrate. On the other hand, plants deficient in nitrogen would contain an excess of carbohydrate circulating in the sap. In the latter case the organic matter, especially carbohydrates, secreted by the plant roots would be higher and attract more nodule bacteria. Invasion of the roots would thus be facilitated in high-carbohydrate plants.

Returning to our own data, how are we to interpret the results obtained? The effect of the nitrogen sources on the distribution of nodules seems to be due to prevention of development of nodules at the points of early invasion of the plant roots. The nodules which did form are typical of those produced by late root penetration.

When mannitol and a nitrogen source are added to the plant cultures, the effect of the latter upon the distribution of the nodules is offset. It thus appears that nitrogen compounds *prevent* the formation of nodules at the points of early invasion, while mannitol with the nitrogen compounds allows nodules to *develop* at the points of early invasion. This observation suggests that the early invading bacteria do not form nodules, because the carbohydrate supply of the plant is insufficient to allow the organisms to develop to the extent necessary to incite nodule formation. MAZÉ states that plants receiving fixed nitrogen will be low in carbohydrate. STROWD found that as the nitrogen available to soy beans was increased, the sugar content of the sap fell off sharply. EATON (**1**) found that as the total sugars and readily hydrolyzable material (hemicellulose, starch, and dextrin) increased in soy beans receiving increasing length of day, the weight of nodules also increased.

The following hypothesis is advanced as an explanation of the results noted. Nitrogen compounds, at least any of the six used, are taken up by the plants, and thus allow the carbohydrates synthesized to be assimilated

at once. If at this time nodule bacteria penetrate the root hairs, they are unable to produce nodules because of the low concentration of sugars in the plant sap. Thus nodules are not formed by the early invaders. As the plant continues to grow it uses up some of the nitrogen source, and no longer has a supply of nitrogen adequate to support its growth at the incipient rate. The carbohydrates increase in the plant sap; and as this continues, a concentration is reached which enables the nodule bacteria penetrating the root hairs at that time to proliferate and incite nodule formation.

Summary

1. A nutrient solution containing agar was used as a substrate for the culture of red clover plants in bottles. Various nitrogen sources were added to the agar in four concentrations, 2, 5, 10, and 20 mg. of nitrogen per bottle. The nitrogenous substances used were: KNO_3, $(NH_4)_2SO_4$, urea, asparagin, clover-seed extract, and yeast extract. In a second experiment, the same nitrogen compounds were used but 0.5 per cent. mannitol was also added. At the time of harvest, the general appearance of the plant and the number, size, and distribution of nodules were noted.

2. The effect of the nitrogen compounds upon the number of nodules showed considerable variability, and the results were not entirely consistent with increasing concentration of the nitrogen compounds.

3. It was found that all of the nitrogen sources used decreased the size of the nodules formed, and that this effect became more in evidence as the concentration of the nitrogen compound was increased. The nitrogen compounds were also found to affect the distribution of the nodules on the roots. In the untreated control, the nodules were situated for the most part on or near the tap-root; a few large or long nodules were present, with the greater number of them of a fair size but round in shape. As compared with this condition, in the presence of the nitrogen compounds the nodules were principally on the secondary roots, and were almost exclusively round and small. This latter condition was attributed to later invasion of the roots by the organism, owing to the presence of the nitrogen compounds. In the series containing both nitrogen source and mannitol, it was found that the nitrogen source no longer affected the distribution of nodules. In these series the nodules were principally on the tap-roots. Increasing concentrations of the nitrogen compounds did, however, decrease the size of the nodules formed.

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MANGANESE AND THE GROWTH OF LEMNA[1]

NORMAN ASHWELL CLARK

(WITH TWO FIGURES)

Since this paper was submitted to the editor, the paper by McHARGUE and CALFEE (PLANT PHYSIOL. **7**: 697–703, 1932) has appeared. As their conclusions are in substantial agreement with the findings reported in this paper in regard to the essential nature of manganese for the growth of *Lemna*, the original manuscript was returned to me and shortened. It should be noted that in these experiments the *Lemna* were grown free from microorganisms, and that therefore the amount of manganese required for maintenance would be expected to be much lower than with the technique of McHARGUE and CALFEE, as was the case.

It has been previously reported (CLARK and FLY **4**) that it was not necessary to add manganese to solutions of purified salts in order to secure good reproduction of *Lemna major*, and that the addition of manganese to an inorganic nutrient medium in which *Lemna* grew well produced some effect upon the vegetation but little upon the rate of reproduction until toxic concentrations were reached. The possibility that extremely small amounts of manganese might be essential to the plants and be present in sufficient quantity even in salts recrystallized several times, together with the failure of many workers to obtain growth in green plants without manganese, particularly of HOPKINS (**6**) with *Lemna minor*, caused the problem to be investigated further.

Lemna major (*Spirodela polyrhiza*) has been grown in these laboratories with inorganic salt solutions for several years. The nutrient solution used as standard consists of magnesium sulphate (24 mg. Mg per liter), potassium nitrate (312.8 mg. K), primary calcium phosphate (16.1 mg. Ca), and ferric chloride (0.6 mg. Fe), adjusted to pH 4.7–4.8 with KOH (**3**). The salts are recrystallized, usually three times. The water is three times distilled, the final distillation being in pyrex glass, and the solution in preparation and use is handled altogether in this glassware. The substitution of quartz Erlenmeyer flasks for pyrex made no change in the rate of growth of the plants. This medium has been used for the growth of *Lemna minor* by ASHBY (**1**) at the University of London. *Lemna* has been found valuable for physiological experiments by BOTTOMLEY and MOCKERIDGE in England, OLSEN in Denmark, and by SAEGER, DEUBER, WOLFE, and others in this country.

[1] Contribution from the Chemistry Department, Iowa State College.

In order to test for the presence of manganese, oxidation by periodate was used in phosphoric acid solution (**7**). It was usually possible to detect as low as 0.001 mg. Mn in 50 cc., and additions of 0.001 mg. caused an alteration in the color. The standard inorganic medium for *Lemna* gave no trace of manganese, indicating less than 0.001 mg. in 50 cc., or a smaller amount than 1 part in 50 million. When a quantity of the salts sufficient to make 20 liters of the standard solution was put into 100 cc. (with the precipitate removed and possible loss checked by adding Mn), the solution contained 0.006 mg. Mn in the 100 cc. The amount of manganese in the standard medium was therefore of the order of 1 in 3000 million. By further purification, solutions were obtained which contained 5×10^{-5} mg. of Mn per liter when the iron was supplied as chloride and 8×10^{-5} with iron as citrate. Experiments were made to determine whether the plants would reproduce at these concentrations, or where the minimum occurred under the conditions of growth.

The material, free from microorganisms (**5**), was grown in 100 cc. of the sterilized medium in Erlenmeyer flasks. Artificial light was supplied for 14.5 hours daily, at approximately 400 foot-candles at the surface of the plants. Temperature was kept at 25°. The plants were transferred twice a week to fresh solutions except where otherwise stated, and their number was reduced as the flasks filled, usually about once a week. The fronds were counted and the rate of reproduction, K, determined for the equation $\log_{10}N - \log_{10}N_0 = K(t - t_0)$ (**2**), where N is the number of fronds and t the days of growth. Cultures were run in duplicate or triplicate, but as these checked, curves are shown for only one flask at each concentration. The pH for the ferric chloride solutions was adjusted to 4.7–4.8, and the ferric citrate solutions to 5.7–5.9.

TABLE I

REPRODUCTION RATE AND MN CONCENTRATION

SOLUTION NO.	IRON SUPPLIED AS FERRIC CHLORIDE		
	MN PER LITER	APPROXIMATE CONCENTRATION	K × 100
	mg.	*p.p.m*	
1	2500×10^{-5}	1:40 million	9.1
2	505×10^{-5}	1:200 "	9.1
3	255×10^{-5}	1:400 "	9.1
4	96×10^{-5}	1:1,000 "	9.1
5	50×10^{-5}	1:2,000 "	9.1
6	28×10^{-5}	1:3,600 "	7.4
7	5×10^{-5}	1:20,000 "	.....

Table I shows the reproduction constant for the ferric chloride solutions. In figure 1 the graphs are plotted and K is the slope of the curve. The manganese was added as manganous chloride except in solution 7, where it was present with the ferric chloride.

The plants in solution 7 reproduced at first, but rapidly got smaller and became brown-tipped. In two weeks they were shriveled and almost lifeless. Solution 6 produced plants of good size but of somewhat light color; the chlorophyll in all the plants receiving iron as chloride was less dense than in the plants with the citrate. This solution, with its 1 part Mn to 3600 million, has rather less manganese than the standard medium formerly used, in which there was about 1 to 3000 million. The increase of the manganese to give 1 part in 2000 million (solution 5) raised the rate of reproduction; but further increase of manganese had no effect, as previously noted by CLARK and FLY (**4**) with still larger quantities of manganese.

Table II gives the reproduction constant for the ferric citrate solutions and figure 2 the curves. The manganese was supplied as before, except for solution 14 where it was present with the citrate.

TABLE II

REPRODUCTION RATE AND MN CONCENTRATION

SOLUTION NO.	IRON SUPPLIED AS FERRIC CITRATE		
	MN PER LITER	APPROXIMATE CONCENTRATION	K × 100
	mg.	*p.p.m*	
8	2508×10^{-5}	1:40 million	11.4
9	508×10^{-5}	1:200 "	11.4
10	258×10^{-5}	1:400 "	11.4
11	99×10^{-5}	1:1,000 "	10.4
12	53×10^{-5}	1:2,000 "	9.4
13	31×10^{-5}	1:3,200 "	7.2
14	8×10^{-5}	1:12,500 "	.....

The maximum rate of reproduction is higher than with ferric chloride. The break comes between 1:400 and 1:1000 million. As the manganese content is made smaller the rate of reproduction drops. The plants in solutions 11, 12, and 13 are green and healthy, but are smaller with decreasing content of Mn in the solution. Plants in solution 14, which contained approximately 1 part of manganese to 12,500 million, grew for some days but became very small and most of the fronds died.

In some cases, after the plants had grown for about five weeks with a change of medium twice a week, the change was altered to once a week. It was thought that the speed of reproduction might decrease, especially

where the manganese was low. No noticeable alteration could be found, as will be seen from solution nos. 6 and 11 in figures 1 and 2.

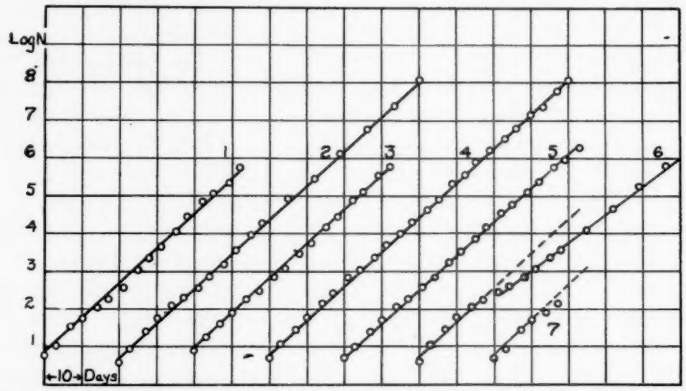

FIG. 1. Reproduction curves with manganese varying. Iron as ferric chloride.

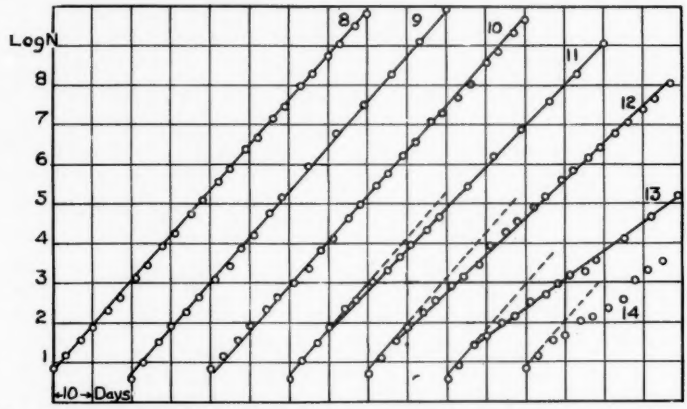

FIG. 2. Reproduction curves with manganese varying. Iron as ferric citrate.

Conclusions

From these results manganese must be classed as essential for the growth of *Lemna major*. Good growth and healthy plants were produced with a concentration of 1 part of manganese in approximately 3000 million under the conditions of the experiments. With iron supplied as ferric chloride at this concentration, the plants were large but somewhat light colored; with ferric citrate the chlorophyll was dense but the plants were slightly smaller. With increased manganese in the solution the rate of reproduc-

tion rose, but soon reached a maximum, after which further additions of Mn had no effect on the rate.

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HENRI LOUIS DUHAMEL DUMONCEAU
1700–1782

BRIEF PAPERS

HENRI LOUIS DUHAMEL DUMONCEAU

(WITH PLATE VIII)

HENRI LOUIS DUHAMEL DUMONCEAU lived at a time when it was possible for a man to be well versed in more than one field of science. He was primarily a physicist, but the desire to apply physics to everyday life has made him better known as an agriculturist, plant physiologist, and plant anatomist. His interest in fruit tree and forest tree growth, fish and fishing, shipbuilding and the methods of sanitation of ocean-going vessels, made him so well known in France that he was made Minister of Marine. His life and works are very briefly reviewed in the Biographie Universelle by DU PETIT-THOUARS, and in Éloge de M. DUHAMEL by CONDORCET.

DUHAMEL was born in Paris in 1700. He was descended from LOTH DUHAMEL, a nobleman of Holland. CHARLES, the son of LOTH DUHAMEL, came to France about 1400 and settled in Denainvilliers. HENRI LOUIS DUHAMEL'S father was ALEXANDER DUHAMEL, Seigneur of Denainvilliers, and his mother was ANNE TROTTIER.

Very little is recorded of his boyhood days. It is known, however, that he made only slight progress in his early studies, except in physics. It is said of him that while he was at the College of Harcourt he retained only one thing, namely, that men in observing nature had created a science called physics.

In order to study this subject he took up lodging close to the Jardin du Roi, the only public institution in Paris at that time where physics was taught. Here he made many friends, some of whom were DUFAY, GEOFFROY ST. HILAIRE, BERNARD JUSSIEU, VAILLANT, and LEMERI. His interest in plant life was aroused by his association with these men, and the study of plants from the point of view of physics became his primary interest. He made such rapid progress in his work that soon he was asked by the Académie des Sciences to solve a difficult problem. A disease reported to be very contagious was attacking the saffron plantations in the Gatinais. Because of its severity the government asked the help of the Académie, and M. DUHAMEL was chosen to make the study. He soon discovered that a fungus in the soil was killing the saffron. As a result of his successful work on this problem he was elected a member of the Académie des Sciences when he was but 28 years of age.

Most of the knowledge developed from the Renaissance to the beginning of the nineteenth century was collected for the glory of the individual investigator and not for the benefit of society. DUHAMEL believed in using

the new truths for the benefit of the country, however, and sought always to better the conditions of the people by his knowledge of physics. His desire to make more truths known prompted him to devote all of his effort toward that end. From the time DUHAMEL was made a member of the Académie des Sciences he devoted nearly all of his time to experimenting and writing on plant physiology. The great services he rendered to agriculture and the science of navigation were a result of this attitude toward knowledge.

He was rather severely upright and modest. As a young man he appeared to be disinterested in people. This and his work kept him from having serious thoughts of a family, and he never married. Except for the time spent in Paris as a student, and later as a member of the Académie and as inspector general of marine, which required some travel, he spent his life with his brother, practicing agriculture.

In 1740, DUHAMEL had become very much interested in meteorology. He began meteorological observations on his land in the Gatinais, and kept detailed records of the weather and its influence upon crop production. Owing to his interest in frost and its effect upon plants, his observations were most intense in the spring and autumn. He attempted to explain the frost hardiness of certain types of fruit trees and the reasons for the lack of hardiness of others. These weather observations, kept throughout the remainder of his life, greatly influenced certain agricultural practices in France.

While he was doing his work in agriculture, plant physiology, and anatomy, LINNAEUS was developing his ideas on systematic botany and laying the foundation for modern nomenclature. STEPHEN HALES had written his "Vegetable Staticks" while DUHAMEL was still a young man, and undoubtedly much of the latter's inspiration and knowledge came from the venerable HALES. By comparison of their works it is obvious that a large part of DUHAMEL'S work was compiled and translated from the works of HALES, MARIOTTE, and MALPIGHI. During his lifetime he contributed more than sixty memoirs to the Académie des Sciences, and he published numerous textbooks which were soon translated into other European languages, largely because of the great knowledge of agriculture included. It has been said that DUHAMEL DUMONCEAU'S greatest contribution to plant physiology was the bringing together of materials from various sources and giving to the world a summary of the work done in the past.

His work on nutrition is not generally accepted. He believed that the digestion of food occurred in the earth and that the leaves produced the suction required to draw the prepared food material into the roots and stems.

Probably the most valuable of DUHAMEL'S work in plant physiology was on the movements of certain plant organs. The periodic movements

of leaves and the curvatures of shoots had already been observed by RAY, who attributed them to changes in temperature. DODART thought that the movements were due to the contractility of fibers on the dry side of the stem. DUHAMEL observed that some of these were heliotropic curvatures caused by light, but that in the case of *Mimosa* leaves the movements took place in darkness as well as in light.

His work on growth followed closely the experiments of HALES, and many of the latter's results were confirmed by new investigations, especially those in which the manner of apical growth in roots was shown. Much of his work on soil, fertilizers, and agricultural crop production was based upon the writings of the English agriculturist, JETHRO TULL. Because of his interest in soils, DUHAMEL made some chemical analyses, and in 1736 he clearly distinguished between the alkalis, potash, and soda.

While DUHAMEL was inspector general of marine he had an opportunity to see the importance of fruit, wheat, and timber to the nation. Owing to his influence, naval construction and sanitary conditions of sea-going vessels were greatly improved. The demand for shipbuilding material caused him to look into the future and to foster tree planting. His interest in forestry, combined with his natural curiosity and desire for useful knowledge, prompted him to study more thoroughly the anatomy and physiology of trees.

From 1755 to 1768 he wrote his best works on vegetable anatomy and physiology, and included practical information on seeding and planting of trees, the growth of wood, its durability, strength, and other physical properties. There are five treatises with a total of nine volumes written on these subjects. The most important of these is "La physique des arbres," published in 1758, in two volumes. These two volumes are a complete treatise on plant anatomy and physiology. They are based upon the works of MALPIGHI, GREW, HALES, and BONNET. The numerous figures and plates, numbering fifty-five, are very well made, and many of them are redrawn from the illustrations in HALES' "Vegetable Staticks."

"La physique des arbres" remained the most complete and most instructive French publication on plant physiology for many decades. It was needed at that time to stimulate further the scientific spirit which was spreading over Europe. It came at the time when PRIESTLEY was beginning his investigations in England and just before INGEN-HOUSZ and LAVOISIER began their famous researches. DUHAMEL'S work was a foundation for many of the important physiological investigations. This was especially true in France, before and after the revolution.

It has been said that DUHAMEL had an instinct for truth in his work on plants, but that he lacked the faculty of combination which is so necessary for thorough investigators in plant physiology. He was an uncritical

compiler as compared with MALPIGHI and HALES; and his biographer, DU PETIT-THOUARS, says that he lacked the ability to distinguish between matters of secondary and those of fundamental importance.

At the age of fifty, DUHAMEL was considered one of the most learned men of Europe. His ardor for study never diminished, but he slowly took on the weight of years and was denied the power to carry on the further scientific work which he had planned. He was honored by being made a member of the Royal Society of London and Dean of the Académie des Sciences before his death. He died in Paris on the 13th day of August, 1782, following a stroke of apoplexy. CONDORCET says of him, "His course, useful, glorious, and peaceful, is one of the happiest that the history of science is able to present."—WARREN W. CHASE, *Division of Forestry, University Farm, St. Paul, Minn.*

NITROGEN CHANGES IN STORED ALCOHOLIC EXTRACTS OF PLANT TISSUES[1]

Most phyto-chemists, when desiring to make an analysis of a plant for nitrogen fractions, choose some procedure using fresh tissue. Some workers, however, still make certain nitrogen determinations on alcoholic solutions, and older literature contains references to a considerable number of such determinations. In order to evaluate such work properly, the writer has made a study of the influence of certain factors on stored extracts of various plants, using an aeration method for estimation of ammonia and the Van Slyke apparatus for amino nitrogen.

Such factors as reaction, time of storage, percentage of alcohol, and presence of minerals were considered. Although the type of material influenced the amount of change, in all but one experiment a continually decreasing percentage of amino nitrogen was found, and conversely an increasing percentage of ammonia. In the tests the concentration of alcohol was found to exert little, if any, influence on the final results. The reaction of the solution was probably the most important chemical factor involved, extremes of both acid and alkali inducing greatest changes. Length of storage is perhaps the dominating factor since these changes are gradual. The most extended test covered two and one-half years, and by this time equilibrium seemed to have been reached in the solution. Some few samples stored for a year also seemed to have reached equilibrium, but these were exceptional.

Summarizing the data presented here, it is evident that under all conditions, and with all types of material investigated, progressive changes

[1] Published with the permission of the Director of the Oklahoma Agricultural Experiment Station.

take place in the nitrogenous fractions when plant materials are stored in alcohol. Since this is true, it is desirable to see whether an explanation of the facts recorded can be found. DAKIN and DUDLEY[2] state that under certain conditions in an acid solution, amino acids will undergo decomposition with the formation of ammonia. An example of this is shown in equation 1.

1. $CH_3CHNH_2COOH = CH_3COCHO + NH_3$

It is also known that under appropriate conditions, amino acids may be deaminized in other ways, two of which follow:

2. $RCH \cdot COOH + H_2O = RCHOHCOOH + NH_3$

$\diagup$
NH_2

3. $RCH \cdot COOH + O = RCHO + NH_3 + CO_2$

$\diagup$
NH_2

If either of two of these equations (1, 3) should represent the reaction taking place, we should be able to substantiate the fact either by finding an increase in ammonia or an increased reducing power. In a few of the experiments recorded there is enough of an increase in ammonia to account for the decrease in amino nitrogen. Generally, however, the decrease in amino nitrogen is greater than can be accounted for by the increased amount of ammonia. In the case of a synthetic solution, the decrease of amino nitrogen was roughly ten times the increase in ammonia.

It should also be possible to check on the increase in reducing power of the solution if the reaction is such as will form a compound having a free aldehyde group (equations 1 and 3). So far, experiments carried out to detect, if possible, changes in reducing power have yielded conflicting results. In pure solutions of sugars and amino acids a slight decrease has appeared, while in natural extracts a very slight increase was noted. Extensive and painstaking work will be necessary to settle this point, for even if there is an increase it will probably be slight and effective over a long period of time.

There is considerable published evidence to the effect that sugars and amino acids can combine under various conditions, even in rather dilute solutions. KOSTYTSCHEW and BRILLIANT[3] report that both amino acids and ammonia react with sugars, chiefly those having aldehyde properties. They also state that the reaction is spontaneous in an alkaline medium. WALD-

[2] DAKIN, H. D., and DUDLEY, H. W. The interconversion of alpha amino acids, alpha hydroxy-acids, and alpha ketonic aldehydes. Part II. Jour. Biol. Chem. 15: 127–143. 1913.

[3] KOSTYTSCHEW, S., and BRILLIANT, W. Die Verwandlungen der Aminosauren in Gegenwart von Zucker. Zeitsher. physiol. Chem. 127: 224–233. 1923.

SCHMIDT-LEITZ and RAUCHALLES[4] report several experiments on the reaction of simple peptids and glucose. They found a rapid reaction at room temperature between the two compounds in alkaline solutions. Various concentrations, such as 1 mol. of glycyl-glycin to 50 mol. of glucose, gave a 37 per cent. combination. Fructose apparently did not react so readily. BORSOOK and WASTENEYS[5] report that there is a reaction between amino acids, peptones, and glucose. At the same time they state that there is no evidence of a synthetic reaction changing the lower fragments into complex derivatives. Neither did they observe the formation of any ammonia, urea, or like compounds. In one experiment they found a decrease in amino nitrogen in slightly acid solution, although the change was not large.

In view of the fact that there is a greater decrease in amino nitrogen than can be accounted for by supposing deaminization, it is well to consider reactions of this type as an explanation of the results reported here. The general equation may be of this type:

$$R \cdot CHO + R'NH_2 = R'N : CHR + H_2O,$$

and can explain the decrease in amino nitrogen without any increase in ammonia. While no direct evidence is advanced to prove that this type of reaction actually occurs, still the knowledge that amino acids and glucose can so react lends some credence to the supposition.

It must be borne in mind that the soluble peptids are probably concerned in the indicated reactions as well as amino acids, since no effort was made to remove them before running the analyses. In fact, evidence has been secured, using pure solutions of glucose and amino acids, indicating that much of the change is due to changes in this polypeptid fraction.—JAMES E. WEBSTER, *Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma.*

CORRELATION OF SPECIFIC HEAT AND PERCENTAGE OF WATER IN APPLE WOOD[1]

(WITH ONE FIGURE)

The use of calorimetry as a means of measuring the water-holding capacity against freezing in colloidal systems originated with THOENES.[2]

[4] WALDSCHMIDT-LEITZ, E., and RAUCHALLES, G. Zur Spezifität der Peptidasen. II. Vergleich der Peptid-zucker-kondensation mit der Wirkungsweise des Erepsins. Ber. deut. chem. Ges. **61**: 645–656. 1928.

[5] BORSOOK, H., and WASTENEYS, H. The interaction of free amino-nitrogen and glucose. Biochem. Jour. **19**: 1128–1137. 1925.

[1] Journal paper no. B55 of the Iowa Agricultural Experiment Station.

[2] THOENES, F. Untersuchungen zur Frage der Wasserbindung in Kolloiden und Tierschen Geweben. Biochem. Zeitschr. **157**: 174–186. 1925.

ROBINSON[3] is responsible for many improvements in the procedure, and at the present time it is probably the most satisfactory method for determining frozen water in fresh tissue.

Briefly the method consists of accurately measuring the change in temperature of a known quantity of water by the addition of the frozen material. The temperature of the frozen sample is read immediately before it is dropped into the water. The ice formed in the tissue greatly increases the heat required to warm the sample, and if the weight of the material is known the free or frozen water may be calculated from the following equation:

$$X = \frac{(T_3 - T_4) - SW(T_2 + T_4)}{80 - \frac{T_2}{2}}$$

In the equation:

X = grams of free water
$(T_3 - T_4)$ = temperature change in water
S = specific heat of material
W = weight of material in gm.
$(T_2 + T_4)$ = total temperature change in sample
T_2 = temperature of sample while frozen
80 = calories of heat per gm. of ice.

The equation given is incomplete, in that corrections are omitted for the influence of the apparatus and room temperature upon the temperature change in the water. A detailed discussion of the method is presented by ROBINSON.[3]

The equation requires the use of the specific heat of the material under experimentation. With a number of samples the additional labor involved in making this determination is no small item, since the specific heat of each sample (determined from its duplicate) should be known for accurate results.

A study of the comparative hardiness of fifteen apple varieties at Iowa State College has shown that this additional determination may be eliminated, and the specific heat calculated directly from the water content of the fresh tissue. Since water has a higher heat capacity than any liquid or solid known, it should be an important factor in a measure of the specific heat of any fresh tissue. Figure 1 indicates that water is the determining factor, and that specific heat is almost perfectly correlated with the percentage of water of the tissue.

The position of the regression line was calculated from 65 observations made on current-season apple shoots taken at intervals throughout the period from May to December, 1931. A positive correlation (between

[3] ROBINSON, WM. Relation of hydrophilic colloids to winter hardiness of insects. Colloid Symposium Monograph 5: 199–218. 1927.

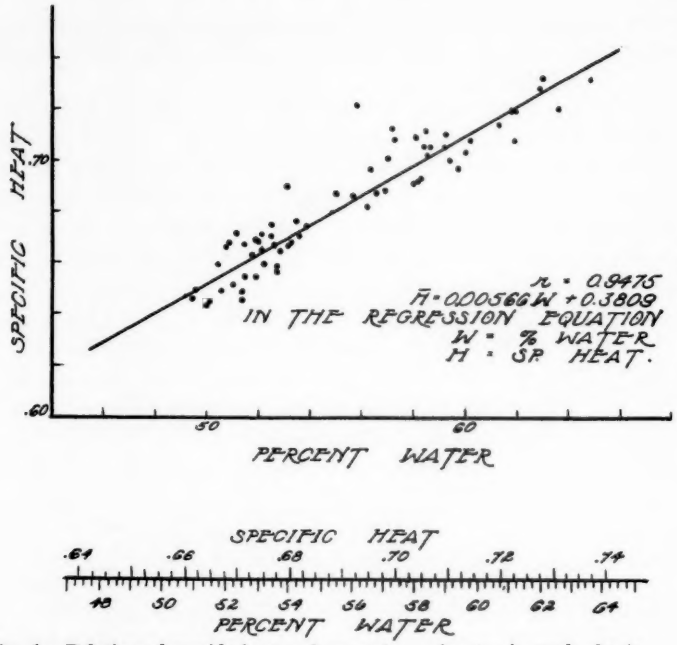

FIG. 1. Relation of specific heat and percentage of water in apple shoots.

specific heat and the percentage of water in the wood) of 0.9475 was obtained in these samples. According to WALLACE and SNEDECOR,[4] a correlation of 0.314 is statistically significant, and the high correlation obtained makes it evident that the specific heat of any sample may be read with more accuracy from the graph than it can be measured from its duplicate. Using the graph, all that is needed to obtain the specific heat of a sample of green apple wood is its percentage of water. In a more convenient form the data may be arranged as shown in the scale below the graph. The value of such an arrangement is obvious when a number of determinations on similar material are to be made.—ARVIL L. STARK, *Iowa Agricultural Experiment Station, Ames, Iowa.*

[4] WALLACE, H. A., and SNEDECOR, G. W. Correlation and machine calculation. Iowa State College Official Pub. 30: no. 4. 1931.

FIELD PROPAGATION OF COTTON BY MEANS OF GRAFTS[1]

Grafting as a method of reproducing cotton asexually first received the attention of the writer in 1930. The results secured from these earlier trials demonstrated the practicability of grafting cotton under greenhouse conditions.[2] The present report gives the results of a grafting experiment conducted during the fall of 1930 to determine the feasibility of grafting cotton plants under field conditions.

The Lone Star variety of cotton was used in this test and the plants had already matured bolls before the grafting was commenced. The saddle-graft method was used on all plants and the individual grafts were made with the minimum lapse of time between cutting the scions and placing them. The stocks remained in the soil with their roots undisturbed in each case.

The field grafting was done on September 18, 19, 21, and 22, under a schedule that provided for work from 1 to 10 P. M. for two of the days, and from 4 to 11 A. M. for the others. A 500-watt lamp was used to illuminate the field for work during darkness. By distributing the grafting throughout the day a wider range of field conditions was introduced. The most important variable was the temperature, which ranged from 68° to 93° F.

After the grafts were completed, they were allowed to remain in the field undisturbed for approximately thirty days. During the development of the unions, the average temperature was 72° F. with an average daily fluctuation of 22° F. In making the final observation, only those grafts that had started stem growth and withstood considerable pressure after the paraffin and twine were removed were considered successful. A total of 220 grafts was made and 87.2 per cent. of them were successful. Also the percentage of successful grafts increased with each additional day's experience. The percentages of successful grafts for September 18, 19, 21, and 22 were 73.3, 88.8, 90.0, and 100.0 respectively. It is quite evident from the results secured that cotton can be grafted successfully under a wide range of field temperatures.—H. E. REA, *Texas Agricultural Experiment Station, Substation no. 5, Temple, Texas.*

[1] Contribution from the Division of Agronomy, Texas Agricultural Experiment Station. Approved by the Director as Technical Contribution no. 228.

[2] REA, H. E. Grafting experiments with cotton. Plant Physiol. 6: 193–196. 1931.

NOTES

Annual Meeting.—As the January number of PLANT PHYSIOLOGY approaches publication, the ninth annual meeting program is in an advanced stage of preparation. The program committee has worked tirelessly to provide an excellent series of meetings. The members of this committee, of which Dr. JOHN W. SHIVE of the New Jersey Agricultural Experiment Station is chairman, deserve great praise for the work they have done. No one who has not been in charge of the preparation of such a program realizes the amount of detail that must receive attention to insure a successful and satisfactory meeting. Atlantic City will provide a high mark for the emulation of future committees.

Barnes Award.—The CHARLES REID BARNES life membership committee, consisting of Dr. W. F. LOEHWING, Iowa, chairman; Dr. C. G. DEUBER, Yale; Dr. R. P. HIBBARD, Michigan State College; Dr. E. S. JOHNSTON, Smithsonian Institution; and Dr. R. S. GIRTON, Purdue, has selected Dr. CHARLES F. HOTTES, Professor of Botany at the University of Illinois, as the 1932 life member. There are now eight members who have been honored by the Society in this manner. The award was established at the Kansas City meeting in 1925, the first award being made at Philadelphia in 1926. A double award was made in 1929.

Dr. CHARLES FREDERICK HOTTES was born at Mascoutah, Illinois, July 8, 1870. His undergraduate work was done at the University of Illinois, and his graduate work began in the same institution. He received his bachelor's degree in 1894, and his M.S. in 1895. Later he studied in Europe, and holds the M.A. and Ph.D. degrees from the University of Bonn. The latter degree was conferred in 1901. He has served the University of Illinois since 1895, except for the period he spent in Europe: assistant in botany 1895–1898; instructor 1901–1902; assistant professor 1902–1913; professor of plant physiology since 1913; and head of the Department of Botany since 1928. He has also been consulting plant physiologist for the Department of Agronomy, and is the only member of the Arts and Science faculty to be also a member of the agricultural faculty in the University. Dr. HOTTES has always been an inspiring teacher, and has distinguished himself in the field of cellular physiology, particularly experimental cytology. He has also shown great ingenuity in the designing of apparatus for the control of environment of plants. The Society has been greatly honored by the committee's action.

Executive Committee.—The Executive Committee of the American Society of Plant Physiologists for 1932–1933 consists of the following members: Dr. DENNIS R. HOAGLAND, Dr. C. O. APPLEMAN, Dr. W. A. GARDNER, and Dr. W. E. TOTTINGHAM, as present and past officers, and Dr. F. B. CHANDLER and Dr. H. R. KRAYBILL of the Minnesota and Purdue sections respectively.

Physical Methods.—The present constitution of this committee is as follows: Dr. EARL S. JOHNSTON, Smithsonian Institution, chairman; Dr. A. L. BAKKE, Iowa State College; Dr. R. B. HARVEY, University of Minnesota; Dr. D. R. HOAGLAND, University of California; and Dr. WILLIAM SEIFRIZ, University of Pennsylvania. It is expected that invitation papers dealing with physical methods of research will be prepared under supervision by the committee, and published in PLANT PHYSIOLOGY from time to time.

Memorial Committee.—This committee has charge of all memorial programs. At present it consists of Dr. F. M. ANDREWS, Indiana University, chairman; Dr. CHARLES F. HOTTES, University of Illinois; and Dr. C. O. APPLEMAN, University of Maryland. This year the memorial program commemorated the centennial of the birth of SACHS. The program was participated in by Section G of the A. A. A. S., the Botanical Society of America, the Phytopathological Society of America, and the American Society of Plant Physiologists. On the program, representing three of these organizations, were three outstanding American botanists. Dr. CHARLES E. ALLEN represented Section G of the A. A. A. S., Dr. DOUGLAS H. CAMPBELL represented the Botanical Society of America (paper read by Dr. J. G. PEIRCE), and Dr. RODNEY H. TRUE represented the American Society of Plant Physiologists. This meeting brings to a close the Society's commemorative program for 1932. The committee in charge of this feature did excellent work and deserves much credit for the splendid program.

Finance Committee.—The Finance Committee has been reappointed with the same personnel as for the last three years: Dr. CHARLES A. SHULL, University of Chicago, chairman; Dr. WALTER THOMAS, Pennsylvania State College; and Dr. BURTON E. LIVINGSTON, Johns Hopkins University. Any one desiring to contribute, either in a large or a small way, to the several funds operated by the Society is invited to communicate with the chairman, or with the secretary-treasurer, Dr. WRIGHT A. GARDNER, Alabama Polytechnic Institute.

Support.—We wish to appeal again to our present members to maintain membership through the financial crisis if at all possible. Or if this is not

possible, we urge that the member try to find some one to take his place on the Society's roll of members. During the past seven years the cost of publishing PLANT PHYSIOLOGY has been cautiously increased as the Society enlarged, until each member now pays for less than he receives. As long as there was active purchase of back numbers, this could be done safely. At present this income is greatly reduced, and it is necessary to depend on current dues and subscriptions for the entire cost. If we are to avoid cutting into our reserves, our plans for volume 8 must consider the possibility of reduction in size of the volume unless the current income remains steady. The editors will make such a reduction if necessitated, but as there are many manuscripts on hand, it would mean delayed publication of those already accepted and the necessary rejection of many good manuscripts. It costs well over $4,000 per year to publish the journal, and maintenance of our membership and subscription lists at their present size is the only means of securing this amount to publish the volume of 1933. PLANT PHYSIOLOGY is meeting a real need in the field of scientific journalism. Your help is requested to maintain it in unimpaired efficiency.

Manuscripts.—It is necessary to remind our contributors that tables and cuts should be used sparingly. It is unfortunate that these two items make up so large a part of the costs of printing. The editor has noted an increase in the percentage of space given to tables and figures in recent papers. The January and April numbers are overloaded in this regard. Please study manuscripts before submitting them, with regard to the possibility of describing the results other than in tabulated form, and with only an occasional graph or photograph. Papers with one or two tables and a photograph or two are not expensive; but when many full-page tables are included in a single paper, the costs become prohibitive. If writers will maintain a sense of responsibility toward the costs of printing, knowing that they can never pay to the journal anything but a fraction of the costs of publishing their own writings, it will be very helpful. If PLANT PHYSIOLOGY were heavily endowed, of course, colored plates could be included and all the tables and cuts required for extensive presentation of work could be accommodated. It seems better at present, however, to work on the theory that good research can be done without overloading the expensive features of publication.

The editor desires to state that it is no longer our policy to accept carbon copies of manuscripts; an original typewritten copy must be supplied.

Minnesota Section.—The Minnesota Section of the American Society of Plant Physiologists has arranged its program for the year 1932–1933 as follows:

Nov.	1, 1932, Winter hardiness in plants.	Dr. S. T. Dexter
Dec.	13, 1932, The effects of certain toxic substances on the catalase activity of plants.	Raymond Landon Warren Chase
Jan.	3, 1933, Gases in trees.	
Feb.	7, 1933, Factors affecting lycopin formation in plants.	Alfred Vogele
March	7, 1933, Drought resistance in forest vegetation.	Dr. Hardy L. Shirley
April	4, 1933, Recent developments in the theory of the toxic action of wood preservatives.	Dr. Henry Schmitz
May	9, 1933, Hardiness in strawberries.	Ernest Angelo

Members of the Society visiting the Twin Cities are invited to meetings of the local section.

Statistical Methods.—The fourth edition of R. A. Fisher's monograph, Statistical Methods for Research Workers, has been received. The third edition was issued in 1930 (Plant Physiol. 5: 440. 1930). This work is one of the best sources of information available for the biometrician. It is designed for use with such numbers of data as are usually obtainable in biological work. The main changes in the fourth edition are as follows: An additional section on the analysis of covariance has been added at the close of Chapter VIII, on the Analysis of variance; and the part played by the covariance has been emphasized in chapters to which this discussion is pertinent, mainly Chapter V. There are a number of changes also in Chapter III, on Distributions, the appendix of which has been entirely rewritten. There are no other changes of consequence. The book is published by Oliver and Boyd, 33 Paternoster Row, London; and the price is 15 shillings net.